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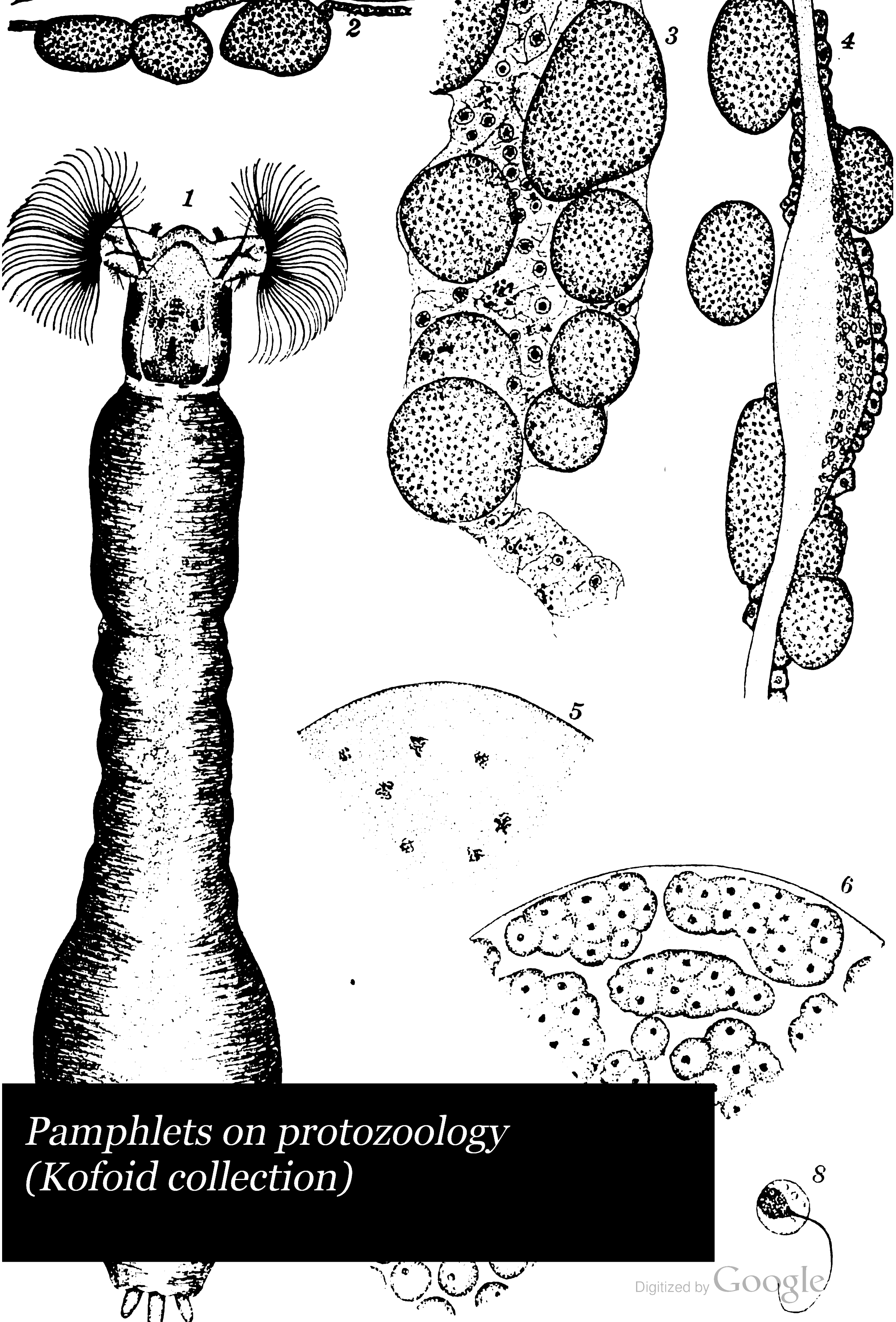

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FACULTÉ DE MÉDECINE DE PARIS

Année 1902 **N° 318**

THÈSE

POUR

LE DOCTORAT EN MÉDECINE

Présentée et soutenue le mardi 6 mai 1902, à 1 heure

PAR

Dimitrie STOICESCU

Né à Ploesti (Roumanie), le 27 juin 1872.

PALUDISME EN ROUMANIE

Notes de statistique et sa prophylaxie

Président : M. BLANCHARD, *Professeur.*
Juges MM. DEBOVE, *Professeur.*
ACHARD *Agrégé.*
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Le Candidat répondra aux questions qui lui seront faites sur les diverses parties de l'enseignement médical

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26, Rue Monsieur-le-Prince, 26
1902

Paras. Prot.

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FACULTÉ DE MÉDECINE DE PARIS

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WURTZ.

Par délibération en date du 9 décembre 1798, l'Ecole a arrêté que les opinions émises dans les dissertations qui lui seront présentées doivent être considérées comme propres à leurs auteurs et qu'elle n'entend leur donner aucune approbation ni improbation.

A MES PARENTS

AVANT-PROPOS

Arrivé au terme de nos études médicales nous saisissons avec empressement l'occasion que nous offre cette thèse pour apporter à nos maîtres, tant de la Faculté que des hôpitaux, le tribut de nos remerciements et de notre reconnaissance. M. le professeur agrégé Schwartz, MM. Giraudeau et Landrieux ont guidé nos premiers pas dans la carrière médicale ; qu'ils trouvent ici l'expression de notre entière gratitude.

Tout le monde dans cette Faculté connait le sens clinique si sûr de M. le professeur Jaccoud, la hauteur de ses vues et l'ampleur de son enseignement magistral ; nous en conserverons toujours un souvenir ému et reconnaissant, ayant eu la bonne fortune d'être son élève pendant notre deuxième année d'externat dans son service de clinique de la Pitié.

C'est M. Babinski, médecin des hôpitaux, qui, pendant notre troisième année d'externat, nous a initié à l'étude des maladies nerveuses ; nous ne saurions trop le remercier pour les connaissances qu'il nous a fait acquérir et pour la grande amabilité qu'il nous a toujours témoignée.

M. le professeur agrégé J. Guiart, avec une bonne grâce dont nous sommes confus, a bien voulu nous aider de ses conseils dans la rédaction de cette thèse et nous a fourni ainsi une aide précieuse ; qu'il nous permette de lui exprimer toute notre sincère gratitude.

Enfin que M. le professeur Blanchard reçoive un témoignage spécial de notre reconnaissance, pour la bienveillance dont il a fait preuve à notre égard en nous faisant l'honneur de présider notre thèse ; par son brillant enseignement, ce maître s'est acquis toute notre respectueuse considération, qu'il veuille bien rester persuadé de notre entier dévouement.

INTRODUCTION

Le paludisme ou fièvre intermittente est une maladie infectieuse d'origine parasitaire. Le paludisme, s'il est inconnu dans les pays froids, est extrêmement répandu par contre dans presque toutes les régions chaudes ou tempérées et augmente de fréquence et d'intensité à mesure qu'on se rapproche de l'équateur. Les pluies, l'humidité du sol, la chaleur, favorisent son développement.

En Europe on le trouve dans presque tous les pays, Ainsi en France il est endémique sur plusieurs points : la Camargue, les Landes, la Charente, la Vendée ou en Sologne, Brenne, Bresse, Dombes et sur les rivages de la Corse. Mais c'est surtout dans la Russie méridionale, en Grèce, Italie, Turquie, Dalmatie, Istrie, Hongrie et Roumanie qu'il sévit et dans ces derniers pays il se présente souvent sous forme de fièvre rémittente grave et pernicieuse.

Les ravages causés par le paludisme sont considérables. Ronald Ross (1) cite pour les Indes le chiffre de 5.026.725 décès pour l'année 1897 seulement. Pour l'Italie Celli donne le chiffre de 15.000. En Roumanie comme nous le verrons dans le chapitre suivant les effets du paludisme sont tout aussi désastreux sinon plus ; aussi de tout temps a-t-on cherché à lutter contre ce fléau. Mais ce n'est que dans ces dernières années, depuis la découverte de son étiologie et de l'agent propagateur, que la prophylaxie du paludisme est devenue vraiment efficace. Les beaux résultats obtenus dans divers pays, notamment en Italie, dans la lutte entreprise contre cette affection nous démontrent la réelle valeur des moyens de défense que nous possédons actuellement et doivent nous faire considérer l'application de ces moyens préventifs comme un strict devoir qui s'impose dans toutes les régions paludiques. Aussi en traitant ce sujet de la prophylaxie paludique, en relatant les dernières expériences et les résultats obtenus, nous avons cherché à mettre en évidence le côté pratique de la question.

Mais avant de traiter la question de la prophylaxie du paludisme, nous avons cru bien faire en donnant quelques renseignements sur la distribution géographique et les ravages causés par le paludisme en Roumanie, tout en regrettant que la saison, peu propice nous ait privé du moyen d'étudier les moustiques de ce pays, avant la fin de ce travail.

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(1) Malaria et moustiques, *Revue scientifique*, 1900, I, p. 769.

PALUDISME EN ROUMANIE (1).

En Roumanie le paludisme est répandu sur presque toute la surface du pays mais c'est surtout dans les régions avoisinant le Danube et dans la région du *Prut* entre autres qu'on trouve de nombreux foyers paludiques.

Par suite de sa conformation géologique, la région du Danube, recouverte d'un très grand nombre d'étangs, marais et marécages, est soumise à de fréquentes inondations. Le delta du Danube et le nord de la *Dobrogea* constituent les principaux foyers paludiques. Cette région très marécageuse, étant très basse, est inondée presque chaque année par les eaux des bras du Danube. D'autre part le déboisement de plusieurs montagnes, fait que les rivières qui y prennent leurs origines sont extrêmement rapides et au moment des grandes pluies sortent de leurs lits, produisent des inondations et contribuent d'autant aux mauvaises conditions hygiéniques de cette région. La grande différence de niveau qui existe pour certaines de ces rivières entre leur point d'origine et le point où elles viennent se déverser dans le Danube contribue encore à favoriser leur débordement.

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(1) La plupart des détails que nous donnons sur le paludisme en Roumanie et surtout les chiffres statistiques, nous les avons empruntés à l'excellent travail de M. le professeur *Fèlix : Istoria Igienei in Romania in sec. XIX si starea ei la inceputul secolului al XX. Bucarest, 1901.*

Une autre cause d'insalubrité de cette région est la grande quantité de terre et sable transportés par le Danube et qui en se déposant dans le delta ralentit le courant des eaux ; les terrains d'alluvions ainsi formés constituent comme on le sait un très bon milieu pour le développement des moustiques.

La plaine du *Prut* et du *Siret* (Moldavie) est recouverte de même d'un grand nombre de marais et marécages.

Les marais et étangs existent encore dans bien d'autres régions.

Ainsi dans le district de *Jassy*, à part les fréquentes inondations des petites rivières qui le traversent, on trouve 181 marais et 128 étangs ; de même le district d'*Ilfov* (1) présente 56 marais et 177 étangs avec une superficie de 103 kilomètres carrés, etc. Aussi les cas de paludisme sont très fréquents dans toutes ces régions marécageuses. Ainsi dans le district d'*Ilfov* (2) avec une population rurale de 242.252 h. il y a eu pendant l'année 1899 sur 46.000 consultations gratuites 8.104 cas de paludisme, à part les cas de paludisme observés à Bucarest et que nous donnerons plus loin.

Dans le district de *Covurlui* (très marécageux aussi), pendant la même année, 2.640 cas sur une population rurale de 81.397 h. Dans le district de *Romanati* (1) pendant l'année 1900, 7.804 paludiques sur une population rurale de 186.519 h. Dans le district de *Muscel* (eau stagnante et étangs) pour 98.730 h. on a donné 9.137 consultations gratuites dont 714 pour des cas de paludisme.

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(1) Dr Stefanescu, *Privire asupra malariei in Romania*, 1898.

(2) *Calauza Sanitara si igienica*, Bucarest, 1900.

Mais de nombreux foyers paludiques existent encore, même dans les régions saines, dépourvues de marécages et à l'abri des inondations. Ainsi à l'hôpital central du district de *Vâlcea* en 1900 sur 1.792 entrants il y a eu 73 paludiques ; dans la ville de *Falticeni* (ville de montagne) en 1900 sur 9.643 h., 367 paludiques. Dans la ville de *Bacau* (15.000 h.) pendant l'année 1898 on a soigné gratuitement 2.433 malades dont 283 paludiques. A l'hôpital rural *Parincea* (Bacau) en 1897 sur 682 entrants, 68 paludiques et sur 7.719 consultations gratuites, 1.163 paludiques.

Le défaut complet de canalisation dans presque toutes les villes de province favorisant l'accumulation et la stagnation des eaux le long des trottoirs et dans les interstices du pavage; l'habitude qu'ont les paysans de certaines communes (districts Argeș et Olt) dépourvues d'eau, de creuser des fosses pour y collecter les eaux de pluies qui leur servent à abreuver le bétail; les barrages qu'ils établissent dans les petites rivières soit pour favoriser la multiplication des poissons soit pour s'en servir comme d'un pont; l'obstruction des fossés qui bordent les routes, etc., sont autant de causes favorables au développement des moustiques et qui nous expliquent très bien la présence des foyers paludiques dans ces régions.

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(1) *Calauza Sanitara si igienica*, Bucarest, 1900.

Maintenant que nous avons vu la répartition du paludisme en Roumanie nous allons donner quelques chiffres qui prouveront la fréquence des cas de fièvres paludéennes, observées pendant un certain nombre d'années.

Voici la statistique des décès dans les villes et les hôpitaux (1).

Pendant l'année 1893 il y a eu 683 décès par paludisme.
— 1894 — 435 —
— 1895 — 366 —
— 1896 — 522 —
— 1897 — 673 —
— 1898 — 527 —
— 1899 — 422 —

Dans la ville de Bucarest comme on le verra par les chiffres suivants, les décès ont de beaucoup diminué depuis la canalisation de la *Dambovitza*, rivière qui traverse la ville :

Pendant l'année 1868 il y a eu 247 décès.
— 1879 — 240 —
— 1875 — 170 —

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(1) M. le professeur Félix fait remarquer dans son travail (*Istoria Igienei in Romania*) qu'il est difficile de se faire une idée bien nette des effets du paludisme en Roumanie par le chiffre des décès seulement; parce que les diagnostics ne sont posés que dans les villes et les hôpitaux, et qu'il n'existe aucune statistique pour les paysans qui meurent dans les villages.

Pendant l'année	1876	il y a eu	112	décès.
—	1877	—	107	—
—	1879	—	97	—
—	1881	—	54	—
—	1890	—	55	—
—	1891	—	72	—
—	1897	—	90	—
—	1898	—	60	—
—	1899	—	98	—

Dans le pays tout entier ont été traités comme paludiques :

Année	Dans les hopitaux	Cons. grat.	Par les méd. d'arrond. et par les méd. de districts.	Médecins de ville	Total
1892	10.272	88.106	79.623	18.513	196.514
1893	8.081	84.950	75.703	22.007	190.241
1894	5.734	72.233	82.843	21.183	181.933
1895	6.367	64.933	53.667	24.094	149.065
1896	5.971	69.294	43.145	30.872	149.282
1897	10.420	116.139	92.021	43.470	250.390
1898	10.736	128.659	73.364	43.614	256.373
1899	6.700	78.176	55.202	38.772	178.860

La recrudescence du paludisme pendant les années 1897 et 1898, est due aux inondations qui ont eu lieu à cette époque.

Dans les infirmeries et les hôpitaux militaires ont été traités comme paludiques :

En 1896	5.940	cas dont	10	décès
1897	8.843	—	4	—
1898	8 831	—	6	—

Ont été exemptés du service militaire pour infirmités dues au paludisme :

En 1893	pour hypertrophie de la rate	2	cachexie palustre	0
1894	—	13	—	28
1895	—	12	—	6
1896	—	13	—	2
1897	—	69	—	5
1898	—	5	—	6
1899	—	29	—	5

Dans les prisons de l'Etat il y a eu en 1896, 413 paludiques dont 2 décès, et en 1897, 353 cas.

On voit d'après ces chiffres combien grands sont les ravages du paludisme en Roumanie.

Maintenant comme prophylaxie on a desséché par ci par là quelques marais, par exemple dans les districts de Ilfov, Ialomitza, Covurlui, etc. ; on a fait des plantations de saules qui, paraît-il, ont donné de très bons résultats ; ainsi dans le district de *Constantza* on plante chaque année de 10.000 à 15.000 saules autour du marais voisin de la ville de *Megidia*. En ce moment on veut dessécher le delta du Danube entre les bras de Saint-Georges et Sulina, ce qui rendrait en même temps à l'agriculture un terrain de 140.000 hectares d'étendue.

Nous devons mentionner aussi les distributions gratuites de quinine, aux paysans paludiques, par les maires des villages. Mais il reste encore beaucoup pour ne pas dire tout à faire pour que le pays soit complètement débarrassé du paludisme.

ÉTIOLOGIE DU PALUDISME

Parasite et moustique.

Il nous est impossible de parler de la prophylaxie du paludisme sans dire quelques mots sur son parasite et sur le moustique qui est l'agent propagateur de cette affection. Nous le ferons d'ailleurs brièvement pour ne pas sortir du cadre de notre sujet.

Le paludisme est dû à un parasite qui rentre dans l'embranchement des *protozoaires*, classe des *sporozoaires*, ordre des *hémosporidies* (1), découvert en 1880 par M. *Laveran* et qui porte son nom, *Hématozoaire de Laveran.*

Avant cette découverte on attribuait le paludisme à des causes diverses : aux germes de l'air (malaria), où aux germes de la terre (tellurisme).

Vers le milieu du XVII^e^ siècle, après que Torti (2), grâce au quinquina, eut différencié cette maladie des autres fièvres avec lesquelles elle était confondue, Morton, Lancisi, Pringle, Baumes observèrent que cette maladie sévissait surtout dans les contrées marécageuses et furent les premiers à émettre la théorie longtemps admise que le paludisme est dû à des miasmes se dégageant du sol ou de l'eau des territoires infectés. En 1799 Rasori et Lancisi attribuent la nocivité de ces miasmes à des animalcules provenant de la décomposition des matières végétales des marais.

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(1) R. BLANCHARD, Instruction à l'usage des méd., des nat. et des voy. *Bulletin de l'Acad. de Méd.*, v. XLIV, p. 7, 1900.

(2) POUCHET, *Leçons de Pharmaco-dynamie*, vol. III.

En 1846 Bassi enseigne que les parasites produisent le renouvellement de l'accès par l'acte de leur reproduction.

Binz en 1867 attribue le paludisme à des organismes inférieurs répandus dans le sang des paludiques. En 1847 Meckel d'abord, Virchow et Frerichs ensuite remarquèrent dans le sang des individus affectés de paludisme la présence d'un pigment noir qu'on appela pigment de la malaria ou *mélanine.*

C'est en étudiant ce pigment que M. Laveran fait sa grande découverte (1880) et démontre que ce pigment est produit dans le corps d'innombrables et minuscules parasites qui vivent dans les globules rouges du sang.

Ce parasite se présente dans le sang sous des formes variées :

Corps sphériques, *corps en rosace, corps en croissant*, rarement *corps flagellés.* L'organisme adulte complètement séparé de l'hématie dans laquelle il se développe prend le nom de *Schizontes.* L'hématozoaire présente deux cycles évolutifs bien distincts :

Un cycle *endogène asexué* (schizogonie) par *sporu-*

lation qui évolue en entier dans le sang de l'homme et qui donne naissance aux *gamètes* qui concourent à la reproduction sexuée ; et un cycle *exogène sexué* qui évolue dans l'estomac des Anopheles. Cette reproduction sexuée porte le nom de *sporogonie* et le résultat de la fécondation, *sporozoïte*. Le *zygote* est le macrogamète fécondé.

Les auteurs italiens admettent trois hématozoaires distincts : Le *Plasmodium malariæ* qui donnerait naissance à la quarte, le *P. vivax* à la tierce et le *P. præcox* à la fièvre pernicieuse, quotidienne, tierce maligne, estivo-automnale ; en France, Laveran, Labbé, Metschnikov, Blanchard considèrent ces formes comme de simples variétés d'évolution d'un même parasite.

MOUSTIQUES. L'agent propagateur du paludisme est le moustique. C'est un diptère, appartenant au sous-ordre des *Nématocères* et il constitue la famille des *Culicidae* (1). Des huit genres que comprend cette famille, seules les différentes espèces du genre *Anopheles* (A. claviger, bifurcatus, A. pseudopictus pour l'Europe, A. Rossii dans les Indes, A. costalis et funestus au Sierra-Leone ; A. Coustanï à Madagascar) sont capables de déterminer l'infection paludique. C'est la femelle de l'Anopheles seule qui transmet la maladie, le mâle étant frugivore.

Les Anopheles présentent de nombreux caractères distinctifs qui permettent leur différenciation d'avec les moustiques du genre Culex qui sont les plus répandus d'ailleurs. Rien qu'en regardant un moustique posé sur un mur on peut distinguer si c'est un Anopheles ou un Culex (1). Le *Culex* a le corps parallèle au mur ou formant avec lui un angle très aigu. L'Anopheles au contraire tient son corps presque perpendiculaire ; de plus, ses pattes sont plus longues et plus grêles et la paire postérieure se relève fortement vers le haut.

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(1) R. BLANCHARD, *loc. cit.* — J. GUIART, Les moustiques, importance de leur rôle en médecine et hygiène. *Annales d'hygiène publique et de médecine légale*, novembre 1900.

La femelle dépose ses œufs à la surface des eaux dormantes ou à faible courant et la larve éclôt le deuxième jour après la ponte. En dehors même d'autres caractères, il est facile de distinguer une larve d'Anopheles d'une larve de Culex rien que d'après sa position dans l'eau. La larve de Culex quand elle respire se tient verticalement par rapport à la surface de l'eau, la tête en bas, de plus elle est très remuante. La larve d'Anopheles flotte horizontalement à la surface de l'eau, reste immobile, ne se déplaçant que rarement.

La larve du Culex se développe dans n'importe quelle eau plus ou moins croupissante, la larve d'Anopheles au contraire se tient de préférence dans les eaux claires, dormantes, épurées par une riche végétation.

Les moustiques tendent à se disperser suivant un plan horizontal s'élevant à peine de quelques mètres au-dessus du sol ; c'est pourquoi les lieux élevés et les étages supérieurs des maisons sont plus salubres que les rez-de-chaussée ; de plus ils craignent les endroits bien ventilés et ne s'éloignent pas beaucoup de leur retraite à moins d'être portés par le vent, et en effet on avait observé depuis longtemps que les foyers paludiques étaient toujours circonscrits et dans les endroits abrités. Ainsi la cité de Norma (1) construite au sommet d'un rocher est très salubre tandis qu'en dessous une autre cité, Ninfa, très florissante au temps des papes, se trouve aujourd'hui complètement dépeuplée, à cause du paludisme qui y sévit. De même à Constantine, à Rome, etc., les quartiers élevés sont épargnés tandis que les quartiers bas sont infestés par les moustiques.

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(1) R. Blanchard, *loc. cit.*

Rôle des moustiques. — Laveran en 1884 avait déjà émis l'hypothèse de la contagion par le moustique par analogie avec ce qui se passe avec la *Filaire Nocturne.* Koch et plus tard Bignami et Mendini émirent cette même théorie en se basant sur des raisons épidémiologiques mais sans donner aucune preuve à l'appui. C'est ***Patrick** Manson* (1894) qui en étudiant l'évolution de l'hématozoaire émit le premier l'opinion d'un cycle extra-humain de ce parasite et c'est sous son impulsion que *Ronald Ross* découvrit finalement en 1898 le rôle joué par le moustique dans la propagation du paludisme, en travaillant sur des oiseaux parasités par l'*Hæmamœba Danilevskyi*, hématozoaire voisin de celui de Laveran. Presque en même temps Grassi, en Italie, remarque la connexité très évidente entre la répartition géographique du paludisme et les Anopheles, qu'il considère comme le seul agent propagateur du paludisme.

Des nombreuses expériences ultérieures faites avec

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(1) A. Celli. Malaria, secondo nuove richerche, 1900.

Bignami, Basti, Anelli, ont confirmé cette observation. Enfin le 22 décembre 1898 Grassi (1) décrit tout le cycle évolutif de l'Hématozoaire de l'homme dans le corps du moustique. Voici en quelques mots ce qui se passe.

Supposons qu'un Anopheles vient de piquer un paludique ; avec le sang qu'il suce l'Hématozoaire pénètre aussi dans son estomac. Une fois là il y a fécondation des *macrogamètes* (éléments femelles) par les *microgamètes* (él. mâles), le produit de la fécondation, *zygote*, pénètre dans la paroi de l'estomac et finalement vient faire saillie à la surface externe de l'estomac sous forme de petits kystes qui au bout d'un certain temps se déchirent et les *sporozoïtes* fusiformes produits par la division des zygotes tombent dans la cavité générale de l'animal, d'où par un procédé inconnu encore, probablement par le courant sanguin, ils arrivent jusque dans les glandes salivaires et de là dans les canaux excréteurs et alors si le moustique vient piquer quelqu'un, avec la goutte de venin qu'il dépose dans la plaie, les parasites pénètrent également.

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(1) Ciclo evolutivo delle semilune nell' Anopheles Claviger ed altri stud. sulla malaria. *Ann d'Igiene Sperimentale* 1899, vol. IX, p. 258.

PROPHYLAXIE DU PALUDISME

Les moyens de défense contre le paludisme ont varié suivant l'opinion qu'on se faisait de l'étiologie de cette maladie. Ainsi Lancisi (1717) attribuant le paludisme qui sévissait dans la campagne romaine aux émanations des Marais-Pontins (1) conseillait la conservation des forêts situées entre ces marais et la ville. On a pratiqué depuis assez longtemps l'assainissement des régions marécageuses, l'expérience ayant prouvé la connexion qui existe entre les foyers paludiques et ces régions ; ainsi les différents travaux du temps des Romains et la Cloaca maxima en particulier n'était autre chose qu'une grande œuvre de drainage et fonctionne encore comme telle aujourd'hui. Lind cite le fait que les habitants de la campagne romaine portaient au cou des sachets d'ail et de camphre pour éloigner les moustiques. La fumée était employée depuis assez longtemps dans le même but et le paysan roumain aujourd'hui encore brûle de la bouse sèche pour éloigner les insectes. Mais ce n'est que depuis la connaissance du rôle que joue le moustique dans la propagation de cette maladie que la prophylaxie paludique est devenue vraiment efficace. Aujourd'hui on sait que le parasite du paludisme ne se trouve que chez l'homme et le moustique, chez l'homme pendant toute l'année, chez le moustique pendant quatre ou cinq mois seulement. Ceci étant connu, tous les efforts tendent d'une part à modifier les conditions favorables au développement des moustiques en assainissant les terrains paludiques et à détruire les Anopheles et leurs larves, agents propagateurs de la maladie ; d'autre part à mettre l'homme à l'abri de leurs piqûres et en guérissant les paludiques de les mettre dans l'impossibilité d'infecter à leur tour les moustiques. Par conséquent, *assainissement*, *destruction des moustiques et larves* et *moyens de protection de l'homme* contre la piqûre des moustiques (moyens mécaniques et moyens thérapeutiques), voilà les trois grands chapitres que nous allons maintenant étudier. Mais avant, nous dirons quelques mots sur l'immunité que présenteraient certaines races à cette affection et sur les essais d'immunisation artificielle tentés en Italie par le professeur Celli, question qui se rattache directement à la prophylaxie du paludisme.

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(1) A. POUCHET, *loco citato*.

Immunité naturelle et essais d'immunisation artificielle.

La question de savoir si certaines races présenteraient une immunité naturelle ou héréditaire a une réelle importance au point de vue prophylactique ; ainsi dans les colonies par exemple on aurait tout intérêt à faire travailler des nègres dans les régions où les blancs ne peuvent pas vivre sans tomber malades. Mais, comme nous le verrons plus loin, aucune race, pas même la race noire ne présente une immunité héréditaire au paludisme ; tout ce qu'on observe, c'est une certaine résistance plus grande à l'infection, due à l'adaptation des populations aux conditions climatériques et miasmatiques locales. Lind (1) et Pringle avaient déjà observé pendant la guerre de Hollande que l'armée anglaise était décimée par les fièvres paludiques tandis que les indigènes restaient indemnes. Boudin (2) avait insisté spécialement sur l'immunité des nègres au paludisme. Mais Laveran (3) dans l'histoire médicale de l'expédition du Niger cite le fait suivant : sur 545 blancs, 130 eurent des fièvres ; parmi les 25 nègres embarqués en Angleterre, 11 cas ; sur les nègres recrutés en Afrique on n'observa aucun cas de fièvres ; ce qui prouve, dit M. Laveran, que cette immunité relative des nègres ne dépend pas de la race mais de leur adaptation aux conditions miasmatiques du pays. Aussi Hirsch (4) note la fréquence du paludisme parmi les nègres d'Abyssinie. L. Colin (5) conteste aussi l'immunité spéciale des noirs et relate que parmi les troupes françaises de l'occupation de Rome on rencontrait comme en Algérie des formes paludiques qui s'observaient rarement parmi les indigènes. Tomasi-Crudeli (1879 et 1882) par contre dit que le degré de résistance individuelle contre le paludisme, est quelquefois héréditaire au point de devenir caractéristique d'une race, il cite les habitants des Abruzzes et de la campagne romaine qui présenteraient une résistance beaucoup plus grande que les autres populations. E. Maurel (1) dit que, parmi toutes les races, les noirs sont ceux qui résistent le mieux et W. H. Welch (2) constate que les noirs hospitalisés de Baltimore présentent une résistance, comparativement avec le blanc, dans la proportion de 1 sur 3.

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(1) A Celli, Sull'immunità dell'infezione malarica, *An. d'Igiene Sperimentale*, IX, 1899.

(2) *Acclimatation des races humaines sur divers points du globe.*

(3) *Diction. encyclop. des sciences médicales*, 1867.

(4) *Handbuch des historisch. geograph. Pathologie Erlangen*, 1860.

(5) *Traité des fièvres intermittentes*. Paris, 1870.

Enfin Plehn et R. Koch (3) observent que les nègres de l'Afrique orientale qui habitent sur la côte paludique ne présentent que des accès de fièvre légère, tandis que les nègres des régions saines qui descendent à la côte, présentent des accès graves tout comme les Européens.

Par conséquent, comme nous l'avons déjà dit au commencement de ce chapitre, aucune race, pas même la race noire, ne présente une immunité congénitale ou héréditaire ; mais les noirs comme les blancs peuvent à la longue acquérir une certaine résistance due à leur adaptation aux conditions climatériques locales.

Immunité artificielle. — A. Celli a fait de nombreuses expériences pour essayer de conférer une immunité artificielle ; mais il a complètement échoué, soit en injectant du sérum sanguin provenant d'un paludique, soit en pratiquant des injections de sucs de différents organes : cerveau, ganglions lymphatiques, rate, moelle osseuse, foie, pancréas. Il a fait aussi des essais d'immunisation médicamenteuse ; en pratiquant sur un cheval des injections intra-veineuses de chlorhydrate de quinine pour obtenir ainsi une sorte de sérum analogue au sérum antidiphtérique, et qu'il injecte ensuite à des individus paludiques ; le résultat a été nul. Il a essayé de même l'iodure de potassium, l'antipyrine, l'acide arsénieux, etc. L'euquinine et le bleu de méthylène sont les seules substances qui lui ont donné des résultats meilleurs.

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(1) *Traité des maladies paludéennes à la Guyane*, 1883.

(2) *A System of Practical med. by American Authors*, 1897.

(3) *Reise Berichte* 1888.

Le seul fait certain qu'il a observé c'est une prolongation de la période d'incubation.

I. ASSAINISSEMENT

L'assainissement comprend une série de travaux capables de modifier les mauvaises conditions hygiéniques d'une région et dans le cas qui nous occupe, la suppression de toutes les causes susceptibles de produire les petites flaques d'eau stagnante qui comme on le sait, constituent l'élément absolument nécessaire au développement des moustiques qui viennent y déposer leurs œufs. Aussi dessécher les mares, les marécages, régulariser la nappe d'eau souterraine qui entretient l'humidité du sol, mettre en mouvement les eaux stagnantes, empêcher les inondations de se produire, maintenir le niveau constant des eaux des lacs et étangs, c'est supprimer toutes les conditions propices au développement des Anopheles et par conséquent à la propagation du paludisme.

Les moyens qu'on a employé sont nombreux : ils dépendent surtout du domaine de l'ingénieur, aussi nous nous contenterons de les indiquer brièvement.

Moyens pour éviter les inondations (1).

a) Les *plantations* sur les montagnes et versants des collines. Pour dévier une partie des eaux de pluie.

b) Les *levées* et *barrages* transversaux, formés par des troncs d'arbres ou en maçonnerie, ne sont plus employés aujourd'hui.

c) L'*endiguement*. Pour empêcher le débordement des rivières et au bord de la mer pour empêcher l'inondation des terres basses dites wattringues.

d) *Bassins et canaux de dérivation*, qui servent à conduire le trop-plein des eaux dans des réservoirs spéciaux et empêcher ainsi leur débordement.

e) *Travaux* pour empêcher l'*érosion* du lit de la rivière.

f) *Curage* des cours d'eaux. Pour empêcher l'enlisement des petites rivières, par les terres qu'elles charrient.

g) *Régularisation* de l'embouchure des petites rivières dans les fleuves et surtout des petits cours d'eau qui débouchent à la mer et qui souvent sont obstrués par les dunes qui se forment sur le rivage; ainsi dans la vallée du Boivre (Loire-Inférieure) on a construit un aqueduc, par dessus lequel la dune a pu se reformer sans dommage. Mais il ne suffit pas d'assurer seulement l'écoulement des eaux, il faut aussi aviser de ne laisser partir que celles qui surabondent et en même temps empêcher l'envahissement des eaux extérieures ; à cet effet on emploie un système dit *porte à bascule* ou *porte mécanique*.

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(1) Celli, *Malaria secondo nuove richerche*, 1900.

Régularisation des lacs (1). — Les eaux de certains lacs subissent de grandes oscillations suivant l'époque de l'année et suivant les années.

Aussi on doit par des barrages et des canaux maintenir leur niveau constant pour empêcher d'une part la baisse des eaux pendant les grandes chaleurs — ce qui constituerait un véritable foyer de moustiques, surtout si le fond est vaseux — et d'autre part les inondations de se produire pendant la crue des eaux.

Dessèchement des étangs. — Lorsque le fond est vaseux et profond, que le terrain situé au-delà de la chaussée de l'étang est bien en contre-bas du fond qu'on veut assécher, le dessèchement d'un étang est assez facile. Il suffit de le mettre à sec et d'ouvrir un fossé de largeur et pente suffisantes pour permettre l'écoulement *rapide* de l'eau.

Puis on assainit le fond par des *drains* pour obtenir un terrain suffisamment résistant et susceptible d'être labouré. C'est ainsi que l'on a desséché les étangs de la plaine du Forez dans le Berry, l'étang du Pujaut (Gard) en 1630, etc.

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(1) Laveran, Projet d'instruction pour la prophylaxie du paludisme. *Bull. Acad. méd.*, XLIII, p. 580, 1900.

Dessèchement des marais. — Deux cas se présentent (1) :

a) Pour les marais dont la disposition des lieux est telle qu'il existe un point inférieur par lequel l'eau puisse trouver une issue permanente ou périodique, le moyen le plus simple est la construction des canaux avec une *pente suffisante* pour permettre l'écoulement rapide des eaux ; mais très souvent il arrive qu'ils soient envahis par une végétation luxuriante qui en ralentissant le courant favorise aussi le développement des moustiques.

b) Comprend les marais dont la surface étant inférieure à tous les points environnants, forme pour ainsi dire une cuvette dont le liquide ne s'échappe que lorsqu'il est arrivé à une hauteur déterminée ; en d'autres termes, les marais dont le fond présente quelques points plus bas que la mer ou les lieux environnants. Dans ce cas, la construction des canaux étant impossible à cause du manque de pente nécessaire on a eu recours à l'épuisement par des machines hydrauliques (p. ex. à Ostia, Marcarese, dans les Landes, Corse, etc.).

Mais pour avoir une bonne amélioration hygiénique il faut régulariser aussi le cours des eaux souterraines.

Eau souterraine. — Pour remédier à l'humidité du sol due à la proximité de la nappe d'eau souterraine et à l'imperméabilité du terrain, le moyen le plus usité et le plus efficace est le *drainage*, qui consiste à creuser des canaux souterrains à parois perméables, remplis d'air, qui fonctionnent comme centre d'attraction pour l'eau du terrain environnant. Ces drains doivent être en nombre suffisant et avec une pente assez prononcée par rapport aux canaux collecteurs ou fleuves où ils se déversent, pour pouvoir empêcher efficacement la formation des marécages.

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(1) Charpentier de Cossigny, *Hydraulique agricole*.

Les premiers drains, appelés dans certains pays aussi, rigoles, gouttières, fosserets, consistaient en un fossé rempli de bois, fascines, quelquefois de pierres et recouvert d'une couche de terre assez épaisse pour permettre le labourage. Dans la campagne romaine (A. Celli) ils sont formés par des grands blocs de tuf disposés de manière à circonscrire une rigole et remplis avec des pierres et du gravier.

Aujourd'hui on pratique le drainage par des grands tubes en terre poreuse qui viennent se déverser dans d'autres tubes plus grands, *drains collecteurs*.

Le drainage a donné de très bons résultats en France, Allemagne, Angleterre, etc., partout où il a été pratiqué.

Dans les régions où sous la couche d'argile imperméable on trouve une couche perméable de cailloux, graviers, galets, on a utilisé dans le même but les *puits absorbants* (boit-tout). On perfore en plusieurs endroits la couche d'argile et le niveau de l'eau baisse, une grande quantité s'écoulant en dessous par ces ouvertures.

Culture intensive. — Tous ces travaux d'assainissement, pour qu'ils soient vraiment efficaces, doivent être complétés par la *culture* qui rend le terrain encore plus salubre en le rendant plus perméable.

La meilleure, est la *culture intensive* qui est aussi la plus rémunératrice. *B. Grassi* (1) fait certaines restrictions à ce sujet. D'après lui la culture intensive réussit toujours à diminuer les cas de paludisme mais seulement dans les régions *dépourvues* d'irrigations, comme par exemple dans certaines régions de l'Italie où l'on a établit des vignes et où depuis les cas de paludisme sont en décroissance et les Anopheles presque disparus. Par contre la culture intensive dans les régions irriguées, comme la Lombardie, non seulement n'a pas diminué les cas de paludisme, mais au contraire a favorisé son développement et celui des Anopheles.

Pour que la culture intensive puisse réussir dans ces dernières régions, il faut que l'eau qui sert à l'irrigation soit *assez rapide* pour empêcher ainsi le développement des moustiques.

Plantations. — Toujours dans un but d'assainissement on a préconisé les plantations d'*eucalyptus*, *pins* et *saules*. Ils agissent grâce à une activité d'absorption par leurs racines qui est en rapport avec l'activité d'évaporation par les feuilles. La croissance rapide de l'eucalyptus et ses propriétés hydrophiles l'indiquent tout spécialement dans les régions où il peut s'acclimater. En faisant ces plantations il faut disposer les arbres de façon à ce qu'il ne puissent empêcher l'insolation du sol et la circulation de l'air. Les eucalyptus ont donné de très bons résultats en Algérie ; de même les plantations de saules en Roumanie et Angleterre.

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(1) *Studi di uno zoologo sulla malaria*, 1900

Déboisement. — Le déboisement dans certains cas a produit une véritable amélioration des conditions hygiéniques en permettant le nivellement du terrain, la construction des canaux, le drainage et la culture.

Conca, Campomonte (Italie) ont été améliorées de cette façon.

Enfin *Grellet* (1) cite la disparition du paludisme du plateau de Châtillon-sur-Loing (Loiret) à la suite de l'incorporation aux terres de culture d'une certaine quantité de chaux (le marnage avait été fait (1840) au point de vue cultural seulement). La culture abondante qui s'en est suivie contribua pour beaucoup probablement à la disparition du paludisme.

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(1) L'influence antimalarique de la chaux. *Rev. Scientifique*, XII^e, n° 17, 1899.

II. DESTRUCTION DES MOUSTIQUES

D'après les nombreuses expériences qui ont été faites à ce sujet il résulte qu'on a tout avantage à détruire le moustique à l'état de larve jeune, car, à cette période de son évolution il résiste beaucoup moins à l'action des différentes substances culicides qu'à l'état adulte (1). Cette manière de procéder présente encore cet avantage, surtout si on opère à la fin de l'hiver ou au commencement du printemps, alors que la nouvelle génération n'est pas encore développée, de pouvoir détruire l'espèce complètement et à peu de frais.

Les substances employées à cet effet sont extrêmement nombreuses.

Elles varient suivant qu'on veut détruire les larves ou le moustique adulte.

a) **Destruction des larves.** — Celli et O. Cassagrandi (1) qui ont étudié de très près l'action culicide d'un grand nombre de substances, considèrent comme inefficace toute substance qui ne tue pas la larve au bout de 72 heures et parmi ces substances il cite :

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(1) Celli e O. Cassagrandi. Per la distruzione delle Zanzare. *Annali d'Igiene sperimentale*, vol XI, 1899, page 317.

Le Permanganate de Potasse n/100 jusqu'à 1 °/₀₀ :

L'Acide Arsénieux en solution ammoniacale à 10°/₀ ;

Le Pétrole à 0,05 centg. pour 100 centim. cub. à la température de 18°.

De même les larves vivent très bien dans l'eau gazeuse, l'eau sulfureuse non saturée (Eau de Tivoli) et dans l'eau de mer.

Parmi les *substances qui tuent les larves* il note que :

Le *permanganate de potasse* à 5 °/₀₀ présente l'action la plus lente. Il ne tue la larve qu'au bout de 72 heures. Le mélange avec HCl est plus actif.

Le sublimé corrosif est plus actif à 1 °/₀₀, tue difficilement la larve, mais ne tue pas la nymphe.

Le lait de chaux à 1 °/₀₀ tue la larve en 72 heures. N'a aucune action sur la nymphe.

L'Eau sulfureuse saturée de So^2 est très active mais très peu pratique. Attaque les objets en fer, détruit la végétation ce qui serait un avantage en désobstruant les canaux, mais en même temps elle devient un danger pour les terrains cultivés, irrigués par cette eau ; de plus elle répand une odeur désagréable et est d'un prix élevé.

Parmi les **substances végétales** les plus actives sont : d'abord *l'infusion de feuilles de tabac*, ensuite, la *poudre de chrysanthème* (qui est soluble dans l'eau). Les autres poudres du commerce sont peu efficaces.

Ces substances ne sont pas toxiques pour les herbivores, mais en revanche elles sont nocives pour certains vers, mollusques et poissons.

Parmi les **substances colorantes**, les plus employées sont le *gallol* et le *vert de malachite*. A la dose de 0,60 °/₀₀ elles tuent en 6 à 12 heures. A la dose minime de 0,0015 elles tuent encore la larve en 48 heures, la nymphe en 101 heures.

En pratique on fait d'abord une solution concentrée qu'on dilue ensuite dans l'eau d'où l'on veut détruire les larves. Pour régler la quantité de solution nécessaire on procède suivant le procédé colorimétrique. Les couleurs d'aniline sont d'un emploi facile. Elles sont très solubles et diffusibles dans l'eau, ne présentent aucun danger pour les plantes herbacées ni pour les terrains cultivés. Leur grand désavantage c'est que en même temps que les larves elles détruisent aussi tous les autres animaux qui vivent dans cette eau, grenouilles, poissons, etc. Quant à leur action sur les mammifères, elle est *nulle* à la dose minime employée pour la destruction des larves.

Ainsi le *gallol* à la dose de 0,20 centigr. n'est pas toxique (pris par la bouche) pour un lapin ou un cobaye de 400 grammes, et à la dose de 0,50 centigr. pour un chien de 3 kilogs. La dose toxique minima pour un lapin est de 0,50 centigr. Cette même dose de 0,50 centigr., chez le chien ne produit que des vomissements (1).

Pétrole. — De toutes les substances employées pour la destruction des larves, c'est le *pétrole* qui a donné les meilleurs résultats. Utilisé en Amérique déjà depuis une cinquantaine d'années, son usage ne s'est généralisé que dans ces derniers temps, à la suite de nombreuses expériences faites un peu partout. Les premiers essais furent peu encourageants (Roberto, Lamborn, Aaron, 1890). Plus tard Howard (1893) réussit à détruire tous les insectes (dans le voisinage de Washigton) en recouvrant 121,92 mètres de superficie avec 68 litres de pétrole. Celli et O. Cassagrandi (1892), après plusieurs expériences conclurent que l'action du pétrole seul n'est pas bien certaine et on a essayé de le rendre plus actif en l'associant avec le *sulfate de fer* (Witfield), avec la *lanoline*, *vaseline* (1), ou avec le *goudron* (2). C'est avec cette dernière substance qu'il a donné de très bons résultats.

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(1) Celli et O. Cassagrandi.

Le pétrole présente le grand avantage sur les autres substances déjà citées, d'être absolument *inoffensif* pour les poissons et les autres animaux qui vivent dans l'eau et de n'exercer aucune action novice sur les mammifères ni sur les plantes. Son emploi s'impose dans toutes les régions infestées par les moustiques et surtout dans les pays qui possèdent des mines de pétrole, à cause de son efficacité et de son faible prix de revient.

L'action du pétrole sur les larves est absolument mécanique.

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(1) Fermi et Lumbau. Tentative di protez. dell'uomo contro le zanzare mediante mezzi chimici. *Annali d'Igiene sperimentale*. vol. xi, 1900.

(2) Laveran. — Au sujet de la destruct. des larves de moust. par huile de pétrole. *C. R. Soc. de biol.*, LII. 1900.

Les larves par suite de la conformation de leur appareil respiratoire ne peuvent pas utiliser l'oxygène de l'eau ; aussi, pour respirer elles sont forcées de venir mettre en contact l'extrémité de leur tube aérien avec la surface de l'eau, alors quelques gouttes de pétrole pénètrent dans l'intérieur de l'appareil respiratoire et produisent ainsi l'asphyxie de la larve.

La dose de pétrole efficace, est de 10 centimètres cubes par mètre carré, on ne tient aucun compte du volume de l'eau. La seul précaution à prendre, c'est que le pétrole fasse une couche continue à la surface de l'eau qu'on veut assainir ; pour cela, il faut le verser lentement et sur plusieurs points différents de la pièce d'eau. Un autre procédé consiste à promener à la surface de l'eau un torchon imbibé de pétrole, fixé au bout d'une perche (1). Le moment propice à cette opération est la fin de l'hiver ou le commencement du printemps alors que les larves sont peu nombreuses et la génération nouvelle pas encore formée. On renouvelle l'opération tous les 15 jours, jusqu'à l'apparition des premiers froids. Pourtant, pendant l'été, à cause des grandes chaleurs qui favorisent son évaporation il serait prudent de renouveler le pétrole tous les 12 jours. Une autre cause qui contribue aussi à diminuer la durée de son action c'est la richesse en matières organiques que présentent certaines eaux paludiques.

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(1) A. Laveran. Projet d'instruction pour la prophylaxie du paludisme. *Bull. Acad. med.* (3), XLIII, n° 581, 1900.

b) **Destruction du moustique parfait.** — La destruction des moustiques adultes se pratique en toute saison. Leur destruction s'impose quand on veut faire une prophylaxie rigoureuse. Après avoir garni les maisons par des toiles métalliques, comme nous l'indiquerons dans le chapitre suivant, il est nécessaire de faire chaque jour le nettoyage complet des chambres pour tuer tous les moustiques qui ont pu s'y introduire furtivement. L'emploi des substances culicides s'impose encore quand on est obligé de passer la nuit en plein air et alors, disons-le tout de suite, le meilleur moyen, quand on ne dispose pas d'une moustiquaire, est de faire un bon feu de paille, la fumée étant un excellent culifuge.

Les substances recommandées pour la destruction, ou plutôt pour l'éloignement des moustiques, sont très nombreuses ; nous n'en citerons que quelques-unes. Weeder (1880) recommande la fumée de feuilles de *mentha pulegium* ou son huile essentielle, Campbell (1891), la fumée de *pyrèthre*, on a proposé encore la *térébenthine*, l'*iodoforme*, le *menthol*, le *camphre*, la *naphtaline*, le *soufre*, la *zanzoline*, etc. Toutes ces substances ne font qu'éloigner ou *endormir* le moustique, rarement elles le tuent, à moins qu'elles n'agissent pendant longtemps et dans un espace bien clos pour que toute l'atmosphère en soit bien imprégnée ; aussi leur emploi en plein air n'a aucun valeur comme culicide. Celli et O. Cassagrandi (1) qui ont expérimenté toutes ces substances, les divisent en trois classes : *subs-*

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(1) *Loco citato.*

tances odoriférantes, *fumée* et *gaz*. Voici le tableau qu'ils donnent relativement à leur action :

a) *Substances odoriférantes* :

	Mort apparente	Mort réelle
Térébenthine	1'	1'
Iodoforme	10'	40'
Menthol	10'	45'
Camphre	4' à 5'	4 à 5 heures
Naphtaline	10' à 35'	8 »
Ail	par moment	48 »

b) *Fumée* :

Fumée de tabac	à l'instant	1' à 3'
Poudre de chrysanthème	5'	1 heure
Feuilles fraiches d'eucalyptus	3' à 5'	3 »
Fumée de bois	par moment	48 »
Poudre de pyrèthres	par moment	5 »

c) *Gaz* :

Anhydride sulfureuse	à l'instant	1'
Ammoniaque	1'	2'

Fermi et Lumbau (1), recommandent comme très actives les *vapeurs de chlore* (quatre à cinq cuillerées de chlorure de chaux sur une assiette et verser dessus 5 à 10 centimètres cubes de So^{4}H^{2}.) Seulement les vapeurs de chlore étant très irritantes, on ne peut employer ce procédé que dans les chambres où on n'a pas besoin d'entrer pendant plusieurs heures, par exemple dans les chambres non habitées pendant le jour.

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(1) Fermi et Lumbau, Liberazione di una citta dalla zanzare. *Annal. d'Igiene Sperimentale*, 1900, vol x.

De cet exposé nous voyons que les meilleures substances culicides et les plus facilement utilisables sont : la fumée de tabac ou de feuilles d'eucalytus et parmi les gaz, les vapeurs de chlore, mais à condition d'opérer en espace clos et pendant un certain temps ; autrement on ne fait qu'endormir les moustiques.

Maintenant que nous avons étudié aussi complètement que possible l'action des diverses substances culicides nous allons relater brièvement les résultats obtenus, en employant ce procédé (destruction des moustiques), par Cl. Ferri et Tonsini (1) dans leur tentative d'assainissement de *l'île Asinaria*. Mais disons-le tout de suite, les résultats très satisfaisants obtenus par Fermi et Lumbau, sont dus en grande partie aux conditions spéciales dans lesquelles ils ont opéré ; autrement par l'emploi *seul* de ce procédé, tout ce qu'on peut obtenir c'est seulement une diminution du nombre des moustiques mais nullement la disparition complète du paludisme.

L'île *Asinaria*, petite île au nord de la Sardaigne, n'est habitée que par un petit nombre d'habitants, par les condamnés et par le personnel du lazaret et de la colonie pénitentiaire. Elle contient plusieurs mares et étangs.

La destruction des larves fut faite par le *pétrole*, du mois de mai jusqu'à la fin de novembre ; la destruction des moustiques libres, par un mélange de poudre de

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(1) La profilassi della malaria e la distruzione delle zanzare nell'isolo dell' Asinaria. *Ann. d'Igiene Sperimentale.* vol x, 1900.

chrysanthème, *pyrèthre*, *valériane* etc., et la zanzoline de Celli et O. Cassagrandi ; dans les dortoirs des condamnés on employa les vapeurs de chlore. En dehors de cela on ne fit usage que des toiles métalliques, fixées aux fenêtres.

Les résultats obtenus furent les suivants ; on ne trouva aucun Anopheles dans aucune des habitations et les Culex pipiens étaient de beaucoup diminués comparativement à l'année précédente. On n'observa aucun cas nouveau de paludisme.

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III. PROTECTION DE L'HOMME CONTRE LES PIQURES DE MOUSTIQUES

a) **Moyens mécaniques.** — La protection de l'homme contre la piqûre des Anopheles est importante à un double point de vue ; d'abord, parce que, en évitant la piqûre, on évite l'infection paludique, ensuite en isolant ainsi les malades on les empêche d'infecter à leur tour les moustiques, propagateurs du paludisme. On arrive au même résultat aussi, par l'emploi des sels de quinine et de l'arsenic, comme nous le verrons dans le chapitre suivant.

Les moustiques, on le sait, pendant le jour restent cachés dans les bosquets, les excavations des arbres, les recoins des habitations ; ils ne font leur apparition qu'après le coucher du soleil. Par conséquent, dans les régions paludiques, *il faut éviter de sortir avant le lever et après le coucher du soleil* ; quand on est forcé de le faire, voici les précautions à prendre : Se couvrir la face et la nuque par un sac en tulle ou en gaze fine, mettre par-dessus un chapeau à larges bords, les extrémités inférieures du voile insérées sous le col du vêtement. Porter des gants et habits assez épais.

Toujours dans le même but on trouve dans le commerce une foule de *pommades* ou *liquides* qui étendues sur la peau ou sur les vêtements, serviraient à éloigner les moustiques. Tous ces produits à base de *vaseline, lanoline, huile de ricin, huile de foie de morue, huile d'amandes amères*, etc., avec *sulfure d'allyle, aldéhyde benzoïque, eau de goudron, eucalyptus*, etc., sont absolument inefficaces (1) pour faire une bonne prophylaxie, leur action étant très faible et d'une durée minime, une à deux heures dans les maisons, une demiheure à une heure à l'air libre.

Toiles métalliques. — L'usage des toiles métalliques aux portes, fenêtres, cheminées, constitue le meilleur moyen prophylactique en empêchant la pénétration des moustiques dans les habitations. Pour être efficaces, les mailles ne doivent pas avoir plus de 2 mm. de côté, fil exclu (2). Elles doivent fermer hermétiquement toutes les ouvertures.

Pour avoir une idée bien nette de ce que c'est qu'une installation de ce genre, de la façon de procéder, et de leur réelle efficacité, nous ne pouvons mieux faire que de relater quelques expériences qui ont été faites à ce sujet, surtout l'expérience d'*Albanella* faite par *Grassi* en 1900.

L'expérience de Grassi (3) 25 mars jusqu'au 30 novembre 1900), a été faite sur les employés de chemin de fer de la ligne qui va de *Battipaglia* à *Poestum* et qui traverse un pays extrêmement paludique. Cette ligne de 20 km. a été divisée en trois tronçons. Sur le *premier tronçon* du premier au cinquième kilomètre, et sur le *troisième* du dix-septième kilomètres jusqu'à *Poestum* on n'a pris aucune mesure de protection sauf le traitement par la quinine de tous ceux qui avaient eu des accès l'année précédente.

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(1) Fermi e Lumbau. Tentavive di protez. dell'uomo contro le zanzare mediante mezzi chimici. *Annali d'igiene sperim.*, 1900. Vol. x.

(2) B. Grassi, *Studi di uno zoologo sulla malaria*, 1900.

(3) B. Grassi, *Rendi cont. della R. acad. dei Lincei*, IX fax. 6, 1900.

Neveu-Lemaire, exposé des expér. du prof. Grassi sur la proph. du paludisme, *Archives de parasitologie*, III, 1900.

Sur la partie de la ligne comprise entre ces deux tronçons, *zone protégée,* d'une étendue de 12 km. comprenant dix maisons et une gare, habitée par cent treize personnes dont trente-trois enfants, on prit les mesures suivantes : Avant l'apparition de la saison des fièvres (26 juin) on a traité, comme sur les deux autres tronçons, tous les anciens paludiques ayant eu des rechutes pendant l'hiver et le printemps et on a garni toutes les maisons par des toiles métalliques. Le 14 juin, Grassi observa les premiers Anophèles infectés et douze jours après (période d'incubation), le 26 juin, constata le premier cas de fièvre dans la région *non protégée.* A partir de ce moment on cesse l'administration de la quinine aux habitants de la zone protégée. Voici maintenant en quoi consistait la protection :

On avait garni toutes les ouvertures des maisons, fenêtes, cheminées par des toiles métalliques à mailles assez fines pour empêcher les moustiques de passer. Doubles portes en toiles métalliques, munies d'un ressort qui les ferme automatiquement. La porte qui fait communiquer la maison avec l'extérieur s'ouvre dans une cage en toile métallique, une sorte de vestibule qui présente à son tour une petite porte basse, munie à sa partie supérieure d'une toile fine qui se tend quand on ouvre la porte et qui a pour but d'empêcher les Anophèles de pénétrer de haut en bas, suivant leur habitude. En dehors de ces précautions, les habitants, après le coucher du soleil, ne sortaient que la figure et le cou protégés par un voile à mailles fines et les mains protégées par des gants épais.

Résultat. Dans la zone protégée aucun cas d'infection récente, dans la zone non protégée, tout le monde plus ou moins atteint de fièvres.

L'Expérience de Mattei (1), faite à la station de *Valsavoja*, endroit fortement paludique, distante de une heure de la station de *Catania*. On a garni les fenêtres et portes de deux chambres par des toiles métalliques fines. Cinq personnes furent soumises à l'expérience parmi lesquelles l'auteur. Ces quatre personnes, des ouvriers, prenaient le train chaque matin pour aller à Catania où ils travaillaient, revenaient le soir à 7 h. 30 et immédiatement après la descente du train s'enfermaient dans leurs chambres, jusqu'au lendemain matin. Pour se protéger, à la descente du train, contre les moustiques, qui étaient très nombreux, ils se badigeonnaient la face et les autres parties découvertes avec de la térébenthine ou avec un savon à la térébenthine. Pour plus de sécurité une fois rentrés chez eux on brûlait de la zanzoline et on visitait minutieusement tous les coins et recoins de l'habitation. Pas de quinine, ni arsenic. L'expérience dura du 7 octobre au 28 novembre et on ne constata *aucun cas de fièvre* parmi les ouvriers ainsi protégés ; les habitants non protégés eurent tous des accès de fièvres, plus ou moins graves.

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(1) La profilassi malarica colla protezione dell' uomo dalle zanzare. *Annal. d'igiene sperimentale*, 1900. v et x.

b) Prophylaxie par moyens thérapeutiques

La quinine a été administrée fort souvent dans un but préservatif, mais les avis exprimés par les auteurs au sujet de son efficacité sont assez discordants. Ainsi le médecin en chef des troupes anglaises qui ont combattu les Ashantees, *Anthyony Home*, regarde comme des plus douteux les résultats de l'administration préventive de la quinine. *Morehead* (1) fait prendre tous les deux jours aux soldats anglais en marche dans les jungles, 0 gr. 13 de sulfate de quinine, pendant une dizaine de jours. Aucun d'eux n'a eu la fièvre, mais il faut dire que la saison paludique était passée. Presque à la même époque, en Espagne, *Valery Meunier*, malgré la distribution de vin de quinquina au personnel d'un chantier de construction d'une voie ferrée, constate que les cinq sixièmes des ouvriers ont des fièvres. *Griesinger* tire la même conclusion défavorable de l'expédition au Niger en 1841. Cependant plus tard, d'après *Boyson* et *Balfour* l'administration préventive de la quinine parut offrir quelque efficacité ; mais dans le voyage de *Livingston* au Zambèse c'est à peine si elle eut quelque influence préservatrice. Si les résultats obtenus, dans ces diverses expériences, ont été peu favorables, cela tient à ce que la dose de quinine administrée était trop faible, pour agir efficacement sur le parasite ; aussi, comme nous le verrons plus loin, à mesure que les auteurs ont perfectionné son mode d'administration, les résultats ont été de plus en plus concluants et actuellement l'administration des sels de quinine est considérée comme un des meilleurs moyens prophylactiques. Ainsi, *Thorel* a pu parcourir les régions les plus insalubres du Mé-Kong sans que ni lui ni ses compagnons contractassent la fièvre, avec 0 gr. 60 à 0 gr. 80 de sulfate de quinine par semaine.

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(1) Longuet, *Semaine médicale*, 1891. Prophyl. des fièvres interm. par la quinine.

A *Asinie* sur la côte ouest de l'Afrique, les officiers de la *Pénélope* faisant une excursion dans la région marécageuse de Tanoë prirent tous du sulfate de quinine à l'exception d'un seul qui refusa d'en prendre confiant dans son immunité ; huit jours après il a été pris de violents accès de fièvre intermittente bilieuse tandis que ses compagnons ont été absolument indemnes. Le fait suivant est cité par *Bryson*. Quatre-vingts matelots envoyés à Siera-Leone pour y travailler, prirent tous de la quinine ; l'officier qui les commandait s'en abstint; seul il a eu la fièvre, etc. *Jilek* (1), de Pola (Istrie) rapporte que: sur 763 soldats logés dans une même caserne, en pays palustre, 500 reçoivent 0 gr. 10 de sulfate de quinine et 18 0/0 seulement ont des fièvres légères avec peu de récidives, tandis que les 230 hommes qui n'avaient pas eu de quinine ont fourni 28 0/0 de paludiques.

L'aide chirurgien *Warren* donne à 200 hommes de

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(1) *The medic. and Surg, History of the War of Rebellion*, 1888.

1900.

son régiment en opérations dans les zones fiévreuses de la Caroline du Sud, 0 gr. 30 de sulfate de quinine par jour, d'avril à octobre 1863 ; ils ne lui fournissent que quatre fièvres intermittentes. Le reste du régiment 3 à 400 hommes soumis à une autre direction médicale, présente plus de 300 cas de fièvres. Pendant la même année 1863 et dans les postes les plus fiévreux du même Etat le chirurgien *Samuel Logan* en administrant 0 gr. 25 de sulfate de quinine par jour à un certain nombre de ses hommes, fait le relevé suivant : 230 sans quinine, fournissent 134 fiévreux soit 58 0/0 ; 246 qui en prennent irrégulièrement, 96 malades, soit 39 0/0 ; enfin 506 qui la prennent régulièrement n'ont que 98 fiévreux, soit 19 0/0. De même les statistiques des médecins américains, qui l'ont largement expérimentée dans la guerre de Sécession, sont très favorables. *Graeser*, (expériences sur bateaux marchands) a obtenu des résultats très satisfaisants avec 1 gr. de quinine pendant trois jours consécutifs, ensuite trois fois par semaine pendant tout le séjour dans la zone dangereuse.

Action de la quinine sur l'hématozoaire et mode d'administration

L'action de la quinine est double ; d'une part elle exalte le pouvoir phagocytaire des leucocytes, d'autre part elle agit directement sur l'hématozoaire. Sous son influence on voit en effet les parasites prendre leurs formes cadavériques ; les flagella surtout, disparaissent rapidement par la médication quinique.

Il paraît, d'après les expériences faites par divers auteurs, que la quinine n'agit pas d'une égale manière sur les différentes formes du parasite.

Gualdi et F. Martirano (1) (Septembre 1899. Hôpital de Santo-Spirito) ont expérimenté sur plusieurs paludiques, dont le sang contenait des hématozoaires aux différents stades de leur développement, pour étudier l'action spécifique des sels de quinine sur le parasite et en même temps pour déterminer si la quinine en circulation dans un sang chargé de corps en croissants, pourrait empêcher leur développement ultérieur dans l'estomac des moustiques qui viendraient piquer les malades à ce moment là.

Voici les conclusions auxquels ils sont arrivés.

a) La quinine administrée à la dose de 2 gr. 50 en une seule fois et ensuite à la dose de 1 gr. à 1 gr. 50 pendant plusieurs jours, *ne fait pas disparaître* les croissants du du sang, quoique l'individu paludique se reconstitue et présente l'apparence de l'homme non infecté.

La cure de quinine par doses intensives, commencée après plusieurs attaques fébriles, *ne prévient pas* la formation des croissants.

b) La quinine circulant dans le sang à *n'importe quelle dose, suffisante pour détruire les parasites amiboïdes*, non seulement ne tue pas les croissants, mais *n'empêche pas même leur développement ultérieur dans l'estomac des Anopheles*.

Les sels de quinine ne *sont actifs que contre les formes*

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(1) L'azione della chinina sulle semilune. *Annali d'Igiene Sperimentale*, 1900, Vol. x.

(1) *Loc. cit.*

amiboïdes des parasites. Les formes parasitaires qui assurent la vie du germe dans le corps du moustique sont réfractaires à la quinine.

Bastianelli, Bignami, Dionisi, émettent la même opinion.

Grassi (1) fait observer que si les croissants persistent en effet pendant longtemps dans le sang des paludiques, toutefois, *10 ou 12 jours après la disparition du dernier accès fébrile, ils perdent la capacité* de se développer dans l'estomac des Anopheles. Une autre remarque que nous devons faire, c'est que les récidives sont à redouter tant que le parasite se trouve dans le sang, même s'il a perdu la faculté de se développer dans l'estomac des Anophèles et d'après les différents auteurs, Grassi, Laveran, *le sang n'est complètement désinfecté qu'après un traitement, continué pendant au moins deux mois après la disparition du dernier accès de fièvre.*

Quant à la dose et au mode d'administration préventive de la quinine ils varient suivant les auteurs ; Koch donne la quinine à *des intervalles éloignés*, un gramme tous les sept ou huit jours. *Grassi*, *Laveran* considèrent cette méthode comme incertaine et préconisent l'administration de la quinine à *la dose journalière de 0 gr. 20* ou *0 gr. 40 tous les deux jours*. L'inconvénient des sels de quinine c'est leur goût désagréable et les différents troubles cérébraux, stomacaux, etc., qui rendent leur emploi prolongé assez difficile.

En résumé, de tout ce que nous avons dit sur la quinine, il résulte : que *son action prophylactique est réelle ; que les sels de quinine, même à faible dose, circulant*

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(1) *Loc. cit.*

dans le sang, empêchent complètement le développement de l'hématozoaire. Par conséquent, dans les régions malsaines, *il faut administrer à tous les individus, pendant la saison paludique* (juin, octobre) *les sels de quinine à dose préventive* de 0 gr. 20 par jour ou 0 gr. 40 tous les deux jours. *Soumettre tous les anciens paludiques* (qui ont eu des accès dans le courant de l'année) *à une longue cure de quinine. Cette cure doit être faite pendant les mois de janvier à juin,* c'est-à-dire avant l'apparition des moustiques ; après avoir ainsi désinfecté le sang des anciens paludiques, en le débarrassant des parasites qu'il contenait et qui constituaient la seule source d'infection paludique durant ces mois, *il faut continuer la quinine à doses purement préventives pendant toute la saison paludique.*

Celli, *Panegrossi* (1), *Conti* (2) recommandent aussi l'*euquinine* qui ne présente pas le goût désagréable de la quinine et n'occasionnerait aucun trouble dans l'organisme.

L'arsenic a été préconisé depuis longtemps dans le traitement de la cachexie palustre (Fowler, 1786 ; au XIX^e^ siècle par Fodéré, Lordat et surtout Boudin ; Kelsch et Kirner (3), Laveran). Son action fébrifuge a été contestée jusque dans ces dernières années. Comme préventif, l'arsenic a été recommandé surtout par *Tomasi-Crudeli.*

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(1) PANEGROSSI, Euchinina e suo valore terapeutico *Gazzetta degli Ospedagli*, 1897, II, p. 1249,

(2) CONTI, L'euchinina nella malaria. *Gazzeta degli Ospedagli*, 1897, II, p. 1447.

(3) *Les maladies des pays chauds.*

A. Celli (1) l'a expérimenté en grand sur les ouvriers de la ligne ferrée qui va de *Riechi* à *Bovino*, localité très palustre. L'arsenic a été administré sous forme de tablettes de gélatine, contenant chacune *un milligr. d'acide arsénieux.* Son action préventive a été très marquée, ainsi : sur les 78 ouvriers soumis à l'expérience, 39 qui n'avaient suivi aucun traitement, furent tous malades ; parmi les 39 autres qui avaient suivi le traitement, il n'y a eu que trois cas de fièvre légère, le reste, ou 36, furent absolument indemnes.

Actuellement on emploie l'arsenic sous forme d'*arrhénal* ou *méthylarsinate sodique*, composé organique de l'arsenic, découvert par M. A. Gautier (2). L'arrhénal a donné de très bons résultats dans le traitement des fièvres palustres et M. *A. Billet* qui l'a expérimenté le considère même comme de beaucoup supérieur aux sels de quinine ; avec une, deux, rarement trois injections hypodermiques (5 centigr. d'arrhénal par dose) il a réussi à guérir complètement treize paludiques qui tous *étaient restés réfractaires à l'action des sels de quinine à haute dose.* Cette médication présente encore le grand avantage sur les sels de quinine, de ne produire *aucun trouble stomacal.* Enfin la déglobulisation du sang qui se poursuit chez les paludiques même sous l'action de fortes doses de quinine, non seulement est arrêtée par l'emploi de l'arrhénal, mais est remplacée par une reproduction rapide des hématies.

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(1) Epidemiologia e la profilassi della malaria secondo le nuovi richerche. *Giornale de R. Soc. ital. d'Igiene*, 1899, vol. x.

(2) La médication arrhénique. Mémoires présentés à l'Acad. de Méd. le 11 et 25 février 1902.

On donne l'arrhénal par la bouche ou en injections hypodermiques, à la dose de 5 centigr. ; dans les cas urgents ont peut aller jusqu'à 15 et même 20 centigr. Après la guérison des paludiques on continue son administration comme préventif à la dose de 5 centigr. (en inject. hypod.) tous les 7 jours comptés à partir du dernier accès.

Expérience d'Ostie

Cette expérience a été faite, l'année dernière (1901) par *Grassi* (1), pour déterminer, d'une part les résultats qu'on peut obtenir *d'une prophylaxie faite par les seuls moyens thérapeutiques*, et d'autre part pour constater les avantages de cette méthode en comparaison avec la prophylaxie par les moyens mécaniques.

L'expérience a été faite à *Ostie*, région très paludique.

Le médicament employé a été un *mélange de quinine*, *acide arsénieux* et *amers* sous forme de pilules pour les adultes, en potion pour les enfants. Ce mélange connu en Italie sous le nom d'*Esanofèle* et *Esanoféline* est fabriqué en grand par *F. Bisleri* (2).

Voici la formule des pilules :

Bichlorhydrate de quinine.	0,10 centigrammes
Citrate de fer............	0,03 —
Acide Arsénieux.........	0,001 milligrammes
Amers..................	0,15 centigrammes

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(1) Per la lotta contro la malaria, *Policlinico* 1901.

(2) J. Guiart, le Paludsime dans la campagne romaine et les récentes expériences du prof. Grassi. (Deuxième campagne antimal. Ostia, 1901.) *Archives de Parasit.* v, fasc. 3, 401, 1902

Quatre-vingt-dix (90) pilules sont nécessaires pour la guérison d'un paludique. La dose journalière est de *six pilules prises le matin en trois fois* (*à 6h, 9h. et midi*) *pour un adulte*

De *quatre pilules* prises dans les mêmes conditions pour un *enfant de 7 à 14 ans ; de deux pilules*, toujours dans les mêmes conditions, pour un enfant de 3 à 6 ans.

Aux enfants en bas âge on donne les mêmes substances mais en solution (Esanoféline) titrée suivant l'âge de l'enfant. Il y a trois solutions:

1° *Pour les enfants de un à deux ans* on donne *180 grammes* de la solution suivante, à la dose journalière de *12 grammes prise en trois fois*, à 6 heures, 9 heures et midi.

Chaque dose contient :

Bichlorhydrate de quinine...	0 gr. 12
Acide arsénieux............	0 gr. 0003
Citrate de fer...............	0 gr. 03
Amers....................	0 gr. 10

2° Pour *les enfants âgés de sept mois à un an*, on donne *120 grammes* de la solution suivante, à la dose de *8 grammes par jour en deux fois*, à 7 heures et à 10 heures du matin.

Chaque dose renferme :

Bichlorhydrate de quinine...	0 gr. 10
Acide arsénieux............	0 gr. 0002
Citrate de fer...............	0 gr. 013
Amers....................	0 gr. 07

3° Aux enfants *âgées de un à sept mois*, on donne

90 grammes à la dose journalière de *6 grammes* en deux fois, toujours le matin.

Chaque dose renferme :

Bichlorhydrate de quinine...	0 gr. 08
Acide arsénieux............	0 gr. 00015
Citrate de fer..............	0 gr. 01
Amers................. ...	0 gr. 05

Voici maintenant la manière dont cette expérience a été conduite et les résultats obtenus.

Le nombre des individus soumis à la cure le 1^er^ juin était de 270 personnes, dont le quart au-dessous de 14 ans, parmi lesquels neuf enfants au-dessous de 4 ans ; très peu parmi eux étaient indemnes de paludisme. Les adultes furent soumis à un traitement intensif de 6 pilules d'*Esanofèle* par jour (prises dans le courant de la matinée), pendant 15 jours consécutifs ; les enfants à une dose correspondante d'*ésanofeline*, pour les guérir de leurs anciens accès. Une fois la cure intensive terminée et les accès disparus, le professeur Grassi, continua à donner l'*Esanofele*, mais à dose pûrement préventive, *d'abord une pilule*, ensuite *deux pilules par jour*, pendant toute la saison malarique.

Les résultats furent extrêmement satisfaisants. Sur les 270 personnes traitées de cette façon, 224 ont été complètement indemnes de fièvre paludique ; 46 ont eu un seul accès de fièvre légère et de peu de durée. Grassi fait observer que ces 46 personnes avaient suivi le traitement d'une façon irrégulière.

Pendant le cours de cette même expérience un certain nombre d'individus furent traités suivant la méthode de

1900.

Koch, par la quinine à longs intervalles; presque tous ont eu la fièvre. De même on constata plusieurs cas de paludisme parmi les personnes protégées uniquement par les toiles métalliques.

Si nous avons insisté un peu longuement sur cette expérience, c'est qu'elle nous a semblé excessivement intéressante par les résultats obtenus, qui nous démontrent qu'on peut acquérir par l'administration rigoureuse et systématique des seuls médicaments une immunité parfaite à l'égard du paludisme.

CONCLUSIONS

I. Le paludisme est extrêmement répandu en Roumanie. Les principaux foyers paludiques se trouvent dans la région avoisinant le Danube, surtout dans le *nord de la Dobrogea* et le *delta du Danube* ; dans la plaine du *Prut* et du *Siret* (Moldavie). En dehors de ces régions il existe encore de nombreux foyers paludiques disséminés un peu partout, dans les districts : d'*Ilfov* (8104 cas de palud. en 1899), de *Covurlui* (2640 cas en 1899), *de Romanatzi* (7804 cas en 1900), de *Jassy*, etc. Les nombreux étangs, marais et marécages (district d'Ilfov, 56 marais et 177 étangs) qui existent dans ces régions et les fréquentes inondations des grandes et petites rivières qui les traversent nous expliquent la présence du paludisme dans toutes ces contrées. Par suite de certaines habitudes locales, favorables au développement des moustiques, on rencontre de nombreux foyers paludiques même dans les régions dépourvues de marais et à l'abri des inondations comme par exemple dans les districts de

Bacau, *Neamtzu*, *Vâlcea*, etc. : de telle sorte que le paludisme se trouve répandu sur presque toute la surface du pays. Les ravages causés sont considérables : ainsi rien que pendant l'année 1898 il y a eu dans tout le pays 256.374 cas de paludisme ; 178.850 cas pendant l'année 1899.

Aussi pour empêcher la propagation du paludisme, maladie éminemment contagieuse, il faut :

II. Traiter tous les anciens paludiques par les sels de quinine, par l'arrhénal (méthylarsinate sodique) ou par le mélange de quinine, arsenic et amers (Esanofèle, Esanoféline).

Cette cure doit être instituée pendant les mois allant de janvier à fin juin, c'est-à-dire avant l'apparition des moustiques

Elle doit être continuée pendant assez longtemps pour les sels de quinine, au moins deux mois après la disparition du dernier accès de fièvre ; pendant 15 jours avec l'ésanofèle (6 pilules par jour pour un adulte) ; pendant quelques jours avec l'arrhénal (5 centigrammes par jour, en injections hypodermiques, ou par la voie stomacale) ; poursuivre ensuite pendant toute la saison paludique : l'administration de la quinine, à dose purement préventive de 20 centigramme tous les jours, ou de 40 centigrammes tous les deux jours, ou l'ésanofèle à la dose de une pilule d'abord, de deux ensuite, par jour ; ou les injections préventives d'arrhénal, 5 centigrammes tous les sept jours comptés à partir du dernier accès.

III. Détruire les larves de moustiques. Leur destruction se fait par le pétrole à la dose de 10 cc. par mètre carré d'eau à désinfecter ; la période propice pour cette opération est la fin de l'hiver et elle doit être renouvelée tous les quinze jours, jusqu'à la fin de la saison paludique.

IV. Garnir par des toiles métalliques toutes les ouvertures des habitations.

Vu : LE PRÉSIDENT DE LA THÈSE,
BLANCHARD.

Vu : LE DOYEN,
DEBOVE.

Vu et permis d'imprimer,
LE VICE-RECTEUR DE L'ACADÉMIE DE PARIS,
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Stolc A.

1900

ROZPRAVY
ČESKÉ AKADEMIE CÍSAŘE FRANTIŠKA JOSEFA
PRO VĚDY, SLOVESNOST A UMĚNÍ.

ROČNÍK IX. TŘÍDA II. ČÍSLO 24.

2

O ZAŽÍVÁNÍ
A
TVOŘENÍ SE UHLOHYDRÁTŮ
U AMOEBOVITÉHO ÚSTROJENCE
PELOMYXA PALUSTRIS GREEFF.

NA ZÁKLADĚ POZOROVÁNÍ A POKUSŮ PODÁVÁ

ANTONÍN ŠTOLC.

(SE DVĚMA TABULKAMI.)

(PŘEDLOŽENO DNE 20. ŘÍJNA 1900.)

V PRAZE.
NÁKLADEM ČESKÉ AKADEMIE CÍSAŘE FRANTIŠKA JOSEFA
PRO VĚDY, SLOVESNOST A UMĚNÍ.
1900.

ROČNÍK IX. TŘÍDA II. ČÍSLO 24.

O zažívání a tvoření se uhlohydrátů
u amoebovitého ústrojence *Pelomyxa palustris* Greeff.

Na základě pozorování a pokusů

podává

Antonín Štolc.

(Se dvěma tabulkami.)

(Předloženo dne 20. října 1900.)

Práci, jejíž výsledky tuto podávám, započal jsem v roce 1892, pokračoval jsem pak v ní po léta následující až do r. 1899, kdy mohl jsem ji teprve ukončiti s výsledkem mne uspokojujícím.

Přiznávám se, že nebyla pro mne snadnou. Byl jsem při ní mimo pobyt na universitě heidelberské odkázán jen na síly své, nemaje k disposici žádné laboratoře veřejné. Již získávání materiálu v dostatečném počtu s místa dosti nepřístupného, (v lesních tůních na Ladví u Ďáblic) bylo obtížným, podlehajíc změnám časovým a jiným překážkám.

Práce má týkala se pole dosud málo vzdělávaného, i bylo třeba vyhledávati method samostatných, k jichž zdárnému provedení nedostávalo se mi někdy prostředků hmotných; bylo třeba vyčkávati, až mohl jsem si jich opatřiti z výdělku denní své mzdy. Pak bylo třeba provésti řadu pokusů dlouhých i namáhavých, jež vyžadovaly času i trpělivosti a přece končily bezvýsledně přivádějíce mne k sráznému úskalí pochyb, až konečně v bludišti otázek a záhad podařilo se mi najíti nit, jež uvedla mne k uspokojivému cíli. Vím, že v rámci práce mé zbývá mnoho ještě důležitých otázek nezodpověděných neb i nedotknutých, a že některé výsledky práce mé neuspokojí přesné vědy, než doznávám, že obojí nedostatečnost pramení ne tak z mé neschopnosti, jako spíše z poměrů, s nimiž zápasiti jest u nás soukromému nemajetnému vědci, donucenému jíti cestou jinou než obyčejnou.

Část práce své vykonal jsem v laboratoři pana prof. O. Bütschliho za pobytu svého v Heidelberce v letním semestru r. 1893. Konám tu milou povinnost i vyslovuji panu professorovi za veškerou mně poskytnutou vědeckou pomoc díky nejupřímnější.

* * *

Snaže se řešiti otázku zažívání a tvoření se uhlohydrátů u Pelomyxy palustris, poznal jsem, že základem této otázky jest výzkum povahy tak zvaných lesklých těles (Glanzkörper, dle jich objevitele Greeffa). Tělesa tato pravidlem kulovitá (někdy až nepravidelná) vyskytují se stále a hojně v těle Pelomyxy i svědčí již toto jich stálé se vyskytování o důležitém úkolu v těle Pelomyxy. *)

Po dlouhé řadě pokusů vyloučil jsem především možnost, že by po stránce chemické lesklá tělesa byla tou neb onou látkou, vyloučiv na př. negativním výsledkem zkoušky Millonovy, jako by byla látkou bílkovinnou. Došel jsem pak poznání, že lesklá tělesa skládají se z blány obalné a z obsahu blanou touto uzavřeného, že pak obsahem jejich jest glykogen a blána obalná jest vytvořena jiným, těžko rozpustným uhlohydrátem.

Obsah lesklých těles jeví řadu vlastností shodných s podstatnými vlastnostmi glykogenu. Uvedu je pořadem zmiňuje se současně k vůli přehlednosti i o vlastnostech blány obalné.

1. Glykogen jeví se býti tělesem beztvárným, sněhově bílým. Lesklá tělesa pozorována drobnohledem jeví se lesklými, sněhově bílými. Bělost ta náleží jejich obsahu, neboť blána obalná, je-li osamocena, jeví se sklovitě průhlednou. Pozorována mikroskopem polarisačním lesklá tělesa nejeví se dvojlomými, nýbrž chovají se jako těleso beztvárné.

2. Glykogen jest ve vodě rozpustný. Obsah lesklých těles rozpouští se ve vodě, blány obalné nikoliv. Při rozpouštění lesklá tělesa nejprve bobtnají zvětšujíce svůj objem. U těles většího rozměru a s tenkou blanou obalnou puká tato blána, obsah tělesa vniká do vody a zůstává na konec jen svraskalá blána. U menších těles a s tlustší blanou obalnou pravidlem blána nepuká, obsah proniká blanou ven do vody a na konec zůstává blána původní podoby.

Rozpouštění obsahu těles lesklých neděje se u všech stejně rychle. To zdá se souviseti jednak s povahou blány obalné, jednak se stavem, v němž obsah lesklých těles se nalézá. Tak nalezl jsem na př., že u jednoho jedince obsah lesklých těles osamocených na sklíčku ve vodě destillované byl vesměs rozpuštěn za $1/4$ hodiny, jindy za $1/2$ hodiny, 2 hodiny,

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*) Pelomyxu nalezl jsem u nás poprvé r. 1889 i podal jsem o tom v »Klubu přírodovědeckém« zprávu provázenou názory namnoze ještě nesprávnými.

12 hodin a konečně až 24. hodiny. Někdy lesklá tělesa u téhož jedince jsou nestejně rozpustna. Tak na př. u jednoho jedince část lesklých těles rozpustila obsah ve 2 hodinách, jiná část (největší) ve 12 hod. a konečně zbytek v 24 hodinách. Lze konečně nalézti lesklá tělesa, jejichž obsah velmi povlovně se rozpouští. Tak pozoroval jsem jedince, u nichž lesklá tělesa rozpustila obsah svůj teprve ve dvou dnech, jindy ve třech i za delší řadu dní, při čemž voda destillovaná byla každých 24 hodin obnovována.

Domněnku, že rychlost rozpustnosti závisí mezi jiným na stavu obsahu lesklých těles, opírám též pozorováním, že obsah rozpouštěl se rychle u těch lesklých těles, u nichž čerstvě byl nahromaděn, kdežto zvolna rozpouštěl se u těles, u nichž čerstvé jeho nahromadění zjištěno býti nemohlo. Čerstvého nahromadění obsahu lesklých těles lze docíliti také pokusmo, o čemž později bude pojednáno.

3. S jodem dává glykogen zbarvení charakteristické. Ke srovnání užíval jsem glykogenu, jehož se mi dostalo z laboratoře prof. Horbaczewského. Na sklíčku isoloval jsem ve vodě destillované tělesa lesklá, od okraje vodního přidal jsem prášku glykogenového a přičinil roztoku jodu v jodidu draselnatém, načež současně glykogen i tělesa lesklá zbarveny byly souhlasně a stejně intensivně, totiž hnědočerveně. Téhož souhlasného a stejně intensivního zbarvení dosáhl jsem, užil-li jsem, ostatek stejným způsobem si počínaje, tinktury jodové. K dalšímu srovnání barvil jsem jodem tělesa lesklá pod krycím sklíčkem ve vodě isolovaná a tu při užití jodové tinktury a při opatrném si počínání mohl jsem konstatovati, že tělesa lesklá zbarvila se napřed vínově červeně a pak dalším pohlcením jodu intensivně hnědočerveně. Jodem barví se jak blána obalná, tak obsah lesklých těles. O tom možno se přesvědčiti na lesklých tělesech, jejichž obsah rychle se ve vodě rozpouští. U takových těles někdy při zbarvování jodem puká blána obalná a obsah vyhrnuje se ven, i možno tu zjistiti, že jak obsah, tak blána obalná jodem jsou zbarveny. Samotné blány obalné, jakýchž nabudeme na př., ponecháváme-li lesklá tělesa s obsahem čerstvě nahromaděným 24 hodiny ve vodě, aby jejich obsah se rozpustil, barví se jodem slabě hnědočerveně.

4. V alkoholu jest glykogen nerozpustný. Lesklá tělesa jsou rovněž v alkoholu nerozpustna, a to jak obsah jejich, tak i blána obalná. O tom přesvědčíme se na př. tímto pokusem: Část lesklých těles téhož jedince dáme na sklíčko do vody destillované, jinou pak část na sklíčko do absolutního alkoholu. Shledáme potom, že ve vodě destillované průběhem 24 hod. obsah veškerých lesklých těles byl rozpuštěn, kdežto tělesa lesklá v alkoholu pozorovaná jeví se i po 24 hodinách vůbec nerozpustna.

5. V hydrátu draselnatém jest glykogen rozpustný (užívá se této vlastnosti jeho při přípravě z materiálu jaterného). Lesklá tělesa jsou v hydrátu draselnatém úplně rozpustna (obsah jejich i blána obalná).

6. V kyselině solné rozpouští se glykogen (této vlastnosti jeho užívá

se rovněž při přípravě z materiálu jaterného). Obsah lesklých těles rozpouští se v kyselině solné, kdežto blány obalné zůstávají nerozpustny.

7. Ve směsi kyseliny dusičné a sírové rozpouští se glykogen (tvoří se dinitroglykogen), rovněž v kys. dusičné samé a v kyselině sírové samé. Ve směsi kyseliny dusičné a sírové, v samé kyselině dusičné a v samé kyselině sírové rozpouští se obsah lesklých těles a zůstávají nerozpustny pouze blány obalné.

8. Kyselina tříslová sráží glykogen z roztoku. Podle toho nutno souditi, že obsah lesklých těles jest v roztoku kyseliny tříslové nerozpustný. Pokusem následujícím přesvědčíme se o správnosti tohoto soudu.

Část lesklých těles z téhož jedince dána jest na sklíčku do vody destillované, jiná část na sklíčku do roztoku kyseliny tříslové. Přesvědčíme se, že obsah lesklých těles ve vodě destillované ve 24 hodinách vesměs se rozpustil, kdežto v roztoku kysleny tříslové obsah i blány obalné těles lesklých pozorovány po 24 hodiny a pak v přestávkách po delších dobách jsou vesměs nerozpustny.

9. Voda barytová sráží glykogen z roztoku (tvoří se barytová sloučenina glykogenu). Tu nutno též souditi, že obsah lesklých těles jest ve vodě barytové nerozpustný. Pokusem následujícím přesvědčíme se o správnosti tohoto soudu.

Lesklá tělesa z téhož jedince, o nichž jsme zjistili, že již ve $^1/_4$ hod. rozpouštějí obsah svůj ve vodě destillované, dána jsou na sklíčko do vody barytové. Pozorována po 24 hod. a v následujících dnech jeví, že obsah jejich i blány obalné jsou ve vodě barytové vesměs nerozpustny.

10. Amoniakalný ocet olovnatý sráží glykogen z roztoku. I tu nutno souditi, že v amoniakalném octě olovnatém bude obsah lesklých těles nerozpustný. Pokus proveden stejně jako s vodou barytovou a shledáno, že obsah i blány obalné lesklých těles jsou v amoniakalném octě olovnatém nerozpustny.

11. Zahříván se zředěnými kyselinami mění se glykogen v cukr (glykosu). Tu nutno souditi, že za týchž okolností táž změna musí se díti s obsahem lesklých těles. Pokus proveden způsobem následujícím.

Vybrán větší počet jedinců Pelomyxy obsahujících četná lesklá tělesa a prostých většinou potravy. Byli pečlivě vypráni ve vodě destillované, pak na sklíčku rozetřeni a dáni do malé zkoumavky. Do té pak přidáno po stejném dílu vody destillované a kyseliny solné, a pak delší dobu opatrně až do varu zahříváno nad kahanem. Po ochlazení tekutiny ve zkoumavce přidáno louhu draselnatého, až tekutina jevila reakci alkalickou, pak přidáno několik kapek roztoku Fehlingova a opět zahříváno, načež objevila se pěkně žlutá sraženina hydrátu mědičnatého, což bylo důkazem, že v tekutině nalezala se látka redukující. Pokus tento není prost výtek. Možno namítnouti, že látka redukující pocházela z plasmatu nebo ze zbytků potravy, nebo konečně z obalu těles lesklých. Přece však positivní výsledek pokusu nabývá ceny ve spojení s uvedenými již shodami a s pozorováním násedujícím.

Možno bylo někdy nalézti jedince úplně prosté potravy, obsahující však četná veliká tělesa lesklá. Když na sklíčku takoví jedinci byli rozetřeni, aby tělesa lesklá byla, pokud možná, isolována, přidána pak byla kyselina sírová a opatrně zahříváno, nabyla tekutina nejprve barvy červené, pak tmavé s odstínem červenavým, až konečně černé. V jednom případě šťastném, kde zbylé plasma obklopovalo větší počet velkých lesklých těles, mohl jsem zjistiti, že zbarvení pocházelo s míst, kde lesklá tělesa se nalézala.

Soudím z tohoto pozorování, že lesklá tělesa zahřívaná s kyselinou sírovou podrobena jsou tým změnám jako uhlohydráty zahřívané s kyselinou sírovou, a že tudíž i po této stránce lesklá tělesa shodují se s uhlohydraty.

12. Enzymy diastatickými, ptyalinem, diastasou pankreasu a diastasou rostlinnou zcukerňován glykogen a proměňuje se v maltosu. Pozoroval jsem lesklá tělesa na sklíčku za temperatury pokoje a zvýšené temperatury v sekretu žlaz slinných, ve vodném i glycerinovém výtažku pankreatu hovězího a v nakyseleném roztoku diastasy rostlinné, vyloučiv při pokusech s výtažkem pankreatu přidáním chloroformu možný účinek bakterií, i shledal jsem průběhem pozorování ve 24 hodinách i po pozorování dalším, že obsah lesklých těles za přítomnosti uvedených enzymů se rozpouští, že však blány obalné zůstávají nerozpustny. Přítomnost maltosy pro nepatrnost předmětu i svízelnost postupu nepodařilo se mně zjistiti.

II.

Z uvedených shod usoudil jsem, že obsah lesklých těles jest glykogen a že blána obalná jest jiný, nesnadněji rozpustný uhlohydrát. Přistoupil jsem pak k pozorováním ukazujícím, jakým změnám podrobena jsou u Pelomyxy tělesa lesklá za jejího života.

Na čerstvém materiálu, jejž přinesl jsem z Ladví v láhvích a dále doma choval, shledal jsem na počátku, že Pelomyxy čile lezly po stěnách majíce podobu válcovitou (přesněji piškotovitou) a chovajíce v plasmatě svém čerstvou potravu a hojná lesklá tělesa, dosahující velikostí svou velikosti jádra a až velikost tuto přesahující. V další době shledal jsem, že Pelomyxy v poměru, jak mizela potrava ve vodě, v níž byly chovány, v pohybování se po stěně nádoby ustávaly a hromadily se v bahně nádoby stávajíce se kulovitými a více méně nehybnými. Plasma v té době obsahovalo u nich málo potravy a lesklá tělesa jevila se menšími. Konečně, obyčejně asi za měsíc od doby, kdy přinesen byl čerstvý materiál, nalezl jsem Pelomyxy vždy v bahně na dně nádoby; byly kulovité, nehybné, barvy bledě bílé, plasma jejich pak neobsahovalo žádné potravy, obsahujíc pouze jádra, soužívné bakterie, shluk zrnek pískových a lesklá tělesa, jež však podstatně byla změněna. Velikost jejich sklesla na dvě až jednu šestinu průměru jádra*), obsah jejich byl úplně vyčerpán, tak že zbyly pouhé průhledné blány obalné, většinou pak v plasmatě nevyskytovala se tělesa tato ojediněle, nýbrž obyčejně větší neb menší jich počet shloučen byl pevně, jakoby spečen v řetízkovité neb různé nepravidelné shluky. Maje za sebou tato pozorování, přistoupil jsem k rozhodujícím pokusům.

Dne 9. října (1893) isolováni dva jedinci do dvou úzkých nádobek s vodou bez potravy. Nádobky dány do velké nádoby s vodou a každá přikryta sklíčkem tak, aby nechány byly malé otvory pro kommunikaci s okolním vodním ústředím. Oba jedinci měli 9. října v plasmatě svém plno lesklých těles, u prvního dosahovala maximálně 10 šestin průměru jádra, u druhého maximálně 20 šestin průměru jádra. Jedinci opětně pod drobnohledem prohlíženi 16. října i shledáno, že rozměry lesklých těles u obou klesly, u prvního dosahovaly maximálně 7 desitin průměru jádra, u druhého 10 šestin průměru jádra. Pak jedinci dále za stejných podmínek isolováni a prohlíženi až 23. října. U obou rozměry lesklých těles jeví se ještě více zmenšeny a zároveň i obsah jejich jeví se úplně vyčerpán. U prvního dosahují lesklá tělesa maximálně 2 šestin průměru jádra, u druhého maximálně $1\frac{1}{2}$ šestiny průměru jádra. První jedinec byv dále za stejných podmínek isolován, prohlížen byl ještě dne 27. října i jevil, že rozměry lesklých těles více neklesly, stále ještě dosahujíce 2 šestin průměru jádra. Z výsledku uvedeného pokusu nutno souditi, že isolovaní jedinci podlehajíce nedostatku potravy donuceni byli sáhnouti k reservní živné látce v plasmatě jejich nastřádané, k tělesům lesklým, následkem čehož obsah lesklých těles byl ponenáhlu vyčerpán a rozměry jejich až na jistou minimální velikost sklesly.

Vedle tohoto pokusu provedl jsem v různých přestávkách řadu jiných pokusů provedených za týmž účelem a vždy provázených stejným výsledkem. Uvedu z nich tyto:

1. Jedinec (v listopadu 1896) isolován stejným způsobem jako dva předešlí. Má na začátku pokusu plno lesklých těles rozměrů maximálně 10 šestin průměru jádra. Po 14 dnech isolace ukazuje lesklá tělesa vyčerpaná a umenšená tak, že jeví maximálně 2 šestiny průměru jádra.

2. Jedinec (26. listopadu 1896) vybrán z dlouhé nádoby, ve které chován materiál přinesený z Ladví. Má hojně lesklých těles naplněných obsahem a dosahujících maximálně 7 šestin průměru jádra. Isolován do dlouhé nádobky, jež před tím naplněna filtrovanou vodou z nádoby, z níž jedinec vyňat. Pak ústí nádobky opatřeno pergamenem, jenž pevně přivázán, načež nádobka ponořena do velké nádoby s vodou. Dne 9. prosince vyňat jedinec z nádobky a prohlížen. Jedinec nalezen bílým, úplně bez potravy a jevil lesklá tělesa, jejichž obsah byl vesměs vyčerpán, při čemž jejich rozměry sklesly, dosahujíce maximálně 5 šestin průměru jádra.

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*) K vůli přehlednosti udávám i dále rozměry lesklých těles podle rozměru jader Pelomyxy.

3. Jedinec (23. dubna 1898) vyhledán v bahně v láhvi, v níž chován byl materiál nalovený v březnu t. r. Má hojně lesklých těles, majících maximálně 3 šestiny průměru jádra, jejichž obsah však není vyčerpán. Isolován jest do dlouhé nádobky stejným způsobem jako předešlý, nádobka pak vnořena do velké nádoby s vodou, v níž byl jedinec vyhledán. Prohlížen za 5 dní jevil se jedinec bílým, bez potravy, a lesklá tělesa měla maximální velikosť 2 šestin průměru jádra a obsah jejich byl úplně vyčerpán.

4. Jedinec (21. května 1898) vybrán z láhve, v níž chován materiál nalovený 12. května t. r. Měl hojně lesklých těles s obsahem nevyčerpaným, dosahujících maximálně 3 šestin průměru jádra. Isolován týmže způsobem jako jedinec předešlý. Prohlížen 24. května jeví se bílým, téměř bez potravy, jeho tělesa lesklá jsou úplně vyčerpána a dosahují maximálně $2^1/_2$ šestiny průměru jádra.

Zjistil jsem, že lesklá tělesa i při úplném vyhladovění nikdy z těla Pelomyxy úplně nevymizejí. Vymizí úplně jejich obsah, však blány obalné zůstávají, třeba umenšeny na minimum. Takové blány obalné umenšené barví se jodem žlutohnědě, což u srovnání s tím, že blány obalné na lesklých tělesech s obsahem čerstvě načerpaným barví se slabě hnědočerveně, svědčí o tom, že v bláně obalné při vyčerpávání obsahu lesklých těles dějí se změny. K úplnému vyčerpání lesklých těles přidružuje se pak jich sestupování se ve skupiny, čili agglutinace, jak jsem mohl zjistiti pozorováním i pokusem.

K agglutinaci lesklých těles přidružuje se i agglutinace jader. Jádra ta, pokrytá bakteriemi tyčinkovitými a obsahující uvnitř krátké a široké klubíčko chromatinové a zřetelné, často veliké kuličky lesklé, seskupují se ve skupiny po dvou, třech až i u značném počtu. Načerpají-li lesklá tělesa znovu obsah, oddělují se opět od sebe po jisté době a totéž děje se i se seskupenými jádry.

Je-li tato agglutinace lesklých těles a jader ve příčinné nějaké souvislosti, zůstává mi záhadou. Možno, že agglutinace ta jest zjevem fysikálně chemickým, že totiž při úplném vyhladovění nastává v plasmatě Pelomyxy jistý výsledný a jednotný stav chemický, při němž jistá látka před tím v plasmatě rozpuštěná se sráží (asi jako při vysolování barev anilinových) a slepuje pak dohromady látkou touto obklopená lesklá tělesa neb jádra. Na možnost tuto ukazuje i ta okolnost, že lesklá tělesa nejsou vždy jen mezi sebou seskupena, nýbrž někdy i s jádry dohromady slepena.

Dále na možnost toho ukazuje i pozdější rozestoupení se lesklých těles i jader, jež vyložiti lze tím, že vyplněním vyčerpaných těles lesklých novým obsahem nastává v plasmatě Pelomyxy jiný výsledný stav chemický, jenž látku sraženou opětně rozpouští a seskupená tělesa lesklá a jádra opět uvolňuje.

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III.

Když zjistil jsem povahu lesklých těles i změny, kterým jsou podrobena průběhem děje životního, přistoupil jsem k řešení otázky, které látky potravní mají vliv na tvoření se lesklých těles, zvláště na tvoření se jich obsahu. Za účelem tím zkoušel jsem tyto látky: 1. uhlohydráty a glykosidy, 2. látky bílkovité, 3. tuky. Při pokusech svých stanovil jsem si pravidlo základní, totiž chovati jedince Pelomyxy, jež k uvedenému účelu jsem zkoušel, v podmínkách pokud možno shodných s oněmi, v jakých v přírodě žijí. Užíval jsem pak materiálu čerstvého z Ladví přineseného, jejž choval jsem v nádobách dlouhých (nazývám je dále dlouhé), jednak materiálu trvalého, jejž pěstoval jsem ve skleněné nádobě velké (nazývám ji dále velkou), tvaru okrouhlého (26 cm. široké a 13 cm. vysoké). V nádobě této byla voda z Ladví z tůně, v níž se Pelomyxy vyskytují, spolu s bahnem, rostlinami a živočichy, kteří společně tam s Pelomyxou žijí. Do této vody přidával jsem denně trochu čerstvé vody říční, v delších pak přestávkách malé kousky gelatiny, části papíru filtrovacího a kusy bavlny.

Pelomyxám stále dobře se tu dařilo, soustřeďovaly se hlavně na rozkládajícím se ve vodě papíru a na bavlně. Ukazovatelem, že Pelomyxám dobře se daří, bylo hojné se vyskytování v téže nádobě oligochaeta z rodu Dero a nálevníka z rodu Spirostoma (při pěstování v zajetí velmi citlivého), kteří v tůních na Ladví jsou stálými průvodci Pelomyxy.

Jedince, jež vybíral jsem z obojího materiálu, isoloval jsem podle potřeby do některé ze tří nádobek skleněných a okrouhlých, jež k vůli stručnosti názývám: krátké (2 cm. široké a $1\frac{1}{2}$ cm. vysoké), dlouhé ($1\frac{1}{2}$ cm. široké a 7 cm. vysoké) a úzké (7 mm. široké a 5 cm. vysoké). Do některé z těchto nádobek dal jsem vodu podle potřeby buď z dlouhých nádob, buď z velké nádoby, vložil jsem tam pipetou jedince, jejž jsem chtěl zkoumati, k tomu jsem přidal látku potravní, kterou jsem chtěl zkoumati, zavěsil jsem pak nebo postavil nádobku do vody do jedné z dlouhých nádob nebo do velké nádoby. Tento způsob měl výhodu, že jedinec i s látkou potravní byl sice isolován, přece však byl ve spojení s ústředím odpovídajícím ústředí přirozenému. K vůli přesnější isolovanosti přikrýval jsem někdy ještě ústí nádobek krycím sklíčkem, nechávaje však při tom malé otvory mezi sklíčkem a okrajem nádobky, aby nebylo přerušeno spojení vody v nádobce s vodou okolní. Dlužno podotknouti, že pokusy v nádobkách samých neb dokonce pod sklíčkem, jak jsem to prováděl v první době svého zkoumání, nedařily se, poněvadž Pelomyxa za těchto podmínek abnormálně se chová a na mnoze brzy hyne.

V následujícím uvádím výsledky svých pozorování a pokusů, jak jsem je shledal na jedincích Pelomyxy krmiv je: A) uhlohydráty a glykosidy, B) látkami bílkovitými a glykogenem, C) tuky.

A) *Uhlohydraty a glykosidy.*

Výzkumy své počal jsem nejprve se *škrobem pšeničním, s různými stavy jeho,* pak *s různými jinými druhy škrobu.* Pozorování a pokusy dály se v nádobkách krátkých neb dlouhých, jež ponořeny byly buď do vody velké nádoby neb do nádob dlouhých; do nádobek isolováni jedinci, jež bylo zkoumati, a k nim přidán nejprve škrob syrový, jehož zrna před tím pečlivým prohlédnutím pod mikroskopem shledána pro účel náš bezvadnými. Krmeno bylo podle potřeby buď celých 24 hodiny neb hodinu, neb jen půl hodiny, pozorování pak dálo se pravidlem za 24 hodin, neb za kratší dobu, neb v různých přestávkách za doby delší. Tu bylo shledáno, že jedinci takto škrobem krmení byli jím, zejména po krmení po 24 hod., tak nacpáni, že ostatní potrava proti tomu téměř úplně zanikala. Na mnohých pak zrnech shledáno, že byla porušena ukazujíce tvary, jež pozorovány byly na zrnech škrobu syrového (s vrstvami koncentrickými), když byla podrobena účinku enzymů diastatických, jak to na př. nověji popsali a vyobrazili Baraneckij na zrnech škrobu pšeničného (Die stärkeumbildenden Fermente in den Pflanzen; Lipsko, 1878) a Arthur Meyer na zrnech škrobu ječmene (Untersuchungen über die Stärkekörner; Jena, 1895).

Vyobrazuji na přiložené tabulce tyto tvary, vzniklé porušením na zrnech škrobových, jimiž byli určitou dobu jedinci Pelomyxy krmeni, jak jeví se za pozorování po 24 hod. neb po delších dobách, nebo po době kratší než 24 hod. Shledal jsem, že tyto tvary na zrnech škrobových vznikají těmito způsoby:

a) Tvořením se kanálků radiárních od obvodu zrna do středu postupujících a rozšiřováním se jich podle rozpustnosti vrstev, jimiž procházejí.

b) Tvořením se výseků postupujících od povrchu do vnitř jádra a rozšiřujících se podle rozpustnosti vrstev, jež znenáhla postupně obnažují.

c) Šířením se puklin ve středu zrna, jež zveličují se podle rozpustnosti vrstev, v nichž se nalezají a jichž se dotýkají.

d) Šířením se obvodní trhliny nalezající se na ostřejším okraji zrna a zveličující se podle rozpustnosti vrstev, v nichž se nalezá a jichž se dotýká.

e) Tvořením se a zveličováním kanálků probíhajících v průběhu jednotlivých vrstev.

f) Celkovým rozpouštěním jednotlivých vrstev, čímž zvrstvení zrna stává se u porovnání se zrnem neporušeným patrnějším, totiž méně rozpouštěné vrstvy vynikají patrněji mezi více rozpouštěnými.

Uvedené způsoby vyskytují se na zrnech ojediněle nebo v kombinacích a týkají se především velkých zrn škrobu pšeničného. U malých zrn shledal jsem, že porušována jsou vesměs jednostejně šířením se radiárních kanálků a střední dutiny, s níž jsou ve spojení.

Podle shody tvarů, jaké jeví syrová zrna škrobová, nalezající se určitou dobu v těle Pelomyxy s tvary, jaké jeví syrová zrna škrobová podrobená vlivu enzymů diastatických, nutno usouditi, že zrna škrobová jsou v těle Pelomyxy stravována a že při tom působí enzym diastatický v těle Pelomyxy vytvořovaný. Mohlo by se namítati, že tu působí bakterie, jež nalezají se hojné v plasmatě Pelomyxy i ve vakuolách potravních, a jež jsem také někdy nalezl ve vakuolách, v nichž byla zrna škrobová, která po delší dobu v těle Pelomyxy se nalezala. Námítku tuto nutno vyvrátiti, neboť nalezl jsem v těle Pelomyxy tvary, jež vznikly porušením zrna škrobového spůsobem nahoře uvedeným, při tom pak nebylo na těchto tvarech při nejbedlivějším pozorování ani stopy bakterií, aniž byly nalezeny ve vakuolách, v nichž některé tyto tvary se nalezaly. Vedle toho provedl jsem následující pokus. Zrna škrobová pšeničná rozdělil jsem na dva díly. Jedním dílem krmil jsem hodinu jedince isolovaného v krátké nádobce, jež byla ponořena do velké nádoby s vodou. Druhý díl dal jsem do jiné krátké nádobky, kterouž jsem ponořil do téže velké nádoby. Obě nádobky, nádobka s jedincem i nádobka se škrobem, ponechány ve velké nádobě 44 hodiny a dále 48 hodin, a po těchto dvou dobách prohlížen současně jedinec i škrob. V jedinci nalezeny byly charakteristické tvary, vzniklé porušením zrn škrobových, na škrobu pak, jenž současně s jedincem v téže vodě se nalezal, pozorovány byly bakterie, které, jak pravdě podobno, mohly se i do těla Pelomyxy dostati a zde podobně působiti. Tu však shledal jsem, že změny, jež bakterie na zrnech tohoto škrobu způsobily, byly docela jiného způsobu než změny, jevící se na zrnech škrobových, v těle Pelomyxy se nalezajících.

Porušování zrn syrového škrobu pšeničného neděje se v těle Pelomyxy za stejnou dobu u všech zrn stejně. Tak na př., krmíme-li jedince Pelomyxy škrobem $^1/_2$ hodiny a potom prohlížíme zrna škrobová v těle Pelomyxy po 24 hodinách, shledáme, že mnohá zrna jsou vůbec neporušena, jiná porušena jsou málo úzkými radiálními kanálky a mělkými výseky, jiná více porušena jsou různým způsobem, hlavně širokými kanálky, a konečně jiná jsou silně provrtána a umenšena na zbytek obklopený vakuolou. Intensivnost porušování zrn škrobových v těle Pelomyxy časem stoupá. Tak na př. krmil jsem více jedinců škrobem 4 hodiny; prohlížením jednoho z nich po $4^1/_2$ hodině shledal jsem, že většina zrn škrobových v těle jeho se nalezajících byla neporušena a jen některá porušena slabě, jiný jedinec prohlížen po 29 hodinách ukazoval již četná zrna v rozličných stupních, slabě až silně, porušená, konečně jiný jedinec, prohlížený až po 6 dnech, jevil proti předešlému zrna porušená v množství mnohem větším a v různých stupních, slabě až silně (až na zbytky značně rozhlodané).

Některá zrna syrového škrobu pšeničného mohou však v těle Pelomyxy dlouhou dobu setrvávati, aniž jsou porušena. Z některých o tom mne přesvědčujících pozorování uvádím následující. Dne 21. listopadu 1894 krmil jsem více jedinců škrobem 24 hodiny, načež choval jsem je ve velké nádobě pečlivě je isoluje do krátké nádobky, do níž jsem přidal něco humusu. Dne 6. dubna 1895 prohlížel jsem jednoho z těchto jedinců a shledal jsem, že v těle jeho nalezají se ještě hojná zrnka škrobová, z nichž některá byla více neb méně porušena, jiná však úplně neporušena.

V předcházejícím zjistili jsme, že zrna syrového škrobu pšeničného jsou v těle Pelomyxy porušována, nastává pak otázka, jak chovají se při tom tělesa lesklá. O tom možno se přesvědčiti pokusem následujícím. Vybrán větší počet jedinců, u nichž vesměs lesklá tělesa měla obsah vyčerpaný a dosahovala maximálně 2 šestin průměru jádra. Jedna část jedinců dána do krátké nádoby se škrobem, druhá část jedinců do jiné krátké nádoby bez škrobu, obě pak současně vloženy na 24 hodiny do velké nádoby blízko sebe. Po 24 hodinách byli obojí jedinci prohlíženi. Jedinci bez škrobu měli lesklá tělesa nezměněná, jedinci krmení škrobem obsahovali v těle svém plno zrn škrobových, z nichž mnohá byla porušena, a jejich tělesa lesklá byla naplněna obsahem a byla zvětšena dosahujíce maximálně 5 šestin průměru jádra.

Z výsledku uvedeného pokusu nutno souditi, že po nakrmení škrobem vyčerpaná tělesa lesklá naplňují se obsahem a zvětšují své rozměry. Vedle uvedeného pokusu provedl jsem dlouhou řadu pokusů jiných, rozličně modifikovaných, vždy však provázených stejným výsledkem, že totiž po nakrmení škrobem vyčerpaná tělesa lesklá naplňují se obsahem a zvětšují se, a že tělesa lesklá již obsahem naplněná rozměry své zvětšují. Nutno ještě něco dodati. Obsah lesklých těles, získaný nakrmením jedinců škrobem, má tytéž význačné vlastnosti (uvedené na začátku naší práce) jako obsah lesklých těles u jedinců, pocházejících z materiálu čerstvě z tůně naloveného. Jest tudíž totožnost povahy obou na jisto postavena.

Uvedu dále k vůli podrobnostem výsledky některých pokusů sem spadajících.

U jedinců, kteří měli před krmením lesklá tělesa vyčerpaná a maximální rozměry $1\frac{1}{2}$ šestiny průměru jádra, shledal jsem, že po 24hodinném krmení tato tělesa vzrostla pravidlem na maximální rozměry 3 šestin průměru jádra, u jedinců, majících před krmením maximální rozměry 2 šestin průměru jádra, že vyrostla po krmení pravidlem na maximální rozměry 5 šestin průměru jádra. U tří jedinců, kteří měli před krmením tělesa lesklá naplněná obsahem a dosahující maximalně velikosti průměru jádra, shledal jsem, že po 24hodinném krmení škrobem měli dva lesklá tělesa zvětšená maximalně na 8 šestin průměru jádra, třetí pak na 9 šestin průměru jádra. U dvou jedinců, z nichž jeden měl před krmením tělesa lesklá naplněná obsahem a dosahující maximálně 7 šestin průměru jádra, druhý 10 šestin průměru jádra, vzrostla tato lesklá tělesa po 24hodinném krmení škrobem u prvního i druhého maximálně na 15 šestin průměru jádra. U jednoho jedince, který měl před krmením lesklá tělesa vyčerpaná a dosahující maximálně 2 šestin průměru jádra, nalezl jsem, že krmen 24 hodiny a pozorován po 3 × 24 hodinách od počátku krmení, měl lesklá tělesa naplněná obsahem a zvětšená maximálně na 10 šestin průměru jádra. Jiný jedinec, jenž měl lesklá tělesa vyčerpaná, maximálních rozměrů $1^1/_2$ šestiny průměru jádra a krmen byl pak 24 hodiny škrobem, měl, pozorován po 18 dnech od počátku krmení, lesklá tělesa naplněná obsahem a dosahující maximálně velikosti průměru jádra, jiný pak jedinec měl za okolností s předešlým stejných lesklá tělesa dosahující maximálně 7 šestin průměru jádra. Též jedince, kteréž jsem isoloval a donutil následkem nedostatku potravy k vyčerpání obsahu lesklých těles a ke zmenšení jejich rozměrů (viz II. časť tohoto pojednání), krmil jsem na konci pozorování 24 hodiny neb déle škrobem a shledal jsem vesměs souhlasně, že lesklá tělesa naplnila se obsahem a vzrostla svými rozměry.

V případech, kde lesklá tělesa jsou v jedincích úplně vyčerpána a většinou agglutinována, zachovávají namnoze tuto agglutinaci i po 24hodinném krmení škrobem, jsou však při tom naplněna obsahem a zvětšena. O tom přesvědčil jsem se několika pokusy i podávám vyobrazení týkající se jednoho takového pokusu. Po době pozdější však se lesklá tělesa vždy od sebe oddělují, jak jsem mohl zjistiti. Na př. po krmení škrobem trvajícím 48 hodin jsou již většinou nespojena a s nimi i též většinou jádra, též před krmením namnoze agglutinovaná.

Hleděl jsem též zjistiti poněkud rychlost, s jakou děje se opětné naplňování vyčerpaných lesklých těles. Jedince, jenž měl lesklá tělesa úplně vyčerpaná, maximální velikosti dvou šestin průměru jádra, krmil jsem hojně syrovým škrobem pšeničným tři hodiny. I shledal jsem, pozoruje jedince hned po třech hodinách, že lesklá tělesa počala se již zřetelně naplňovati obsahem a zároveň již zřetelně byla zvětšena.

Chci ještě zmíniti se o vakuolách, které vyskytují se kolem zrn škrobových v těle Pelomyxy se nalezajících. Mám za to, že vyskytování se zřetelných vakuol kolem zrn škrobových závisí na množství rozpustného materiálu vycházejícího ze škrobových zrn. Neporušená zrna škrobová jsou v těle Pelomyxy plasmatem těsně objata. Zrna mechanicky porušená, všelijak nalomená, jsou většinou objata vakuolami, což lze vysvětliti tím, že obnažením vnitřních vrstev zrna škrobového dána jest enzymu možnost intensivnějšího působení. Zrna porušená působením enzymu jsou v těle Pelomyxy buď bez zřetelných vakuol, buď jsou objata zřetelnými vakuolami. V případě prvním mám za to, že jest tu menší množství rozpustného materiálu vyrobeno než za stejnou dobu v případě druhém, a že téměř současně, jak se materiál rozpustný tvoří, jest v plasma vstřebáván, čímž nedojde k vytvoření zřetelné vakuoly. Rozpustnost škrobu lze zvětšiti tím, že zrna škrobová (v našem případě pšeničná) nabobtnáme působením horké vody, neb že připravíme z nich škrobový maz.

Krmíme-li 24 hodiny jedince Pelomyxy takovým škrobem nabobtnalým nebo mazem škrobovým, jejž jsme před tím nechali vyschnouti a potom zpráškovali, shledáme, prohlížíme-li jedince hned po 24 hodinách, že obsahuje v těle svém veliké množství nabobtnalých zrn škrobových neb částic mazu škrobového a že oboje jsou vesměs v zřetelných vakuolách.

Pozorujeme-li takto nakrmené jedince delší dobu, nalezneme, že objem nabobtnalých zrn škrobových i částic mazu škrobového stále se zmenšuje, při tom však umenšuje se i objem vakuol; později nalézáme zbytky škrobové v těsných vakuolách neb vůbec bez vakuol, konečně pak jen zbytky bez vakuol jodem více se nebarvící. Zde nutno mi podotknouti, že zrna syrového škrobu pšeničného, když v těle Pelomyxy podrobena byla působení enzymu a porušena byla na míru nejkrajnější, jevíce se jako nepatrné provrtané zbytky, ještě jsou jodem charakteristicky zbarvována jako zrna neporušená. To jest ve shodě s pozorováním Baraneckého týkajícím se působení diastasy na syrová zrna pšeničná a svědčí o tom, že i v těch zbytcích stále ještě přítomna jest rozpustnější modifikace škrobová, na níž závisí charakteristické zbarvení jodem.

Z uvedeného pozorování, týkajícího se nabobtnalého a vařeného škrobu pšeničného, možno též souditi, že jest v těle Pelomyxy ztravován. O tom rozhodně se přesvědčíme pozorováním změn na tělesech lesklých. Shledáme, že podobně jako po krmení škrobem syrovým též po krmení škrobem nabobtnalým neb vařeným vyčerpaná tělesa lesklá naplňují se obsahem a zvětšují se, tělesa lesklá již obsahem naplněná že se též zvětšují.

Přesvědčil jsem se o tom řadou pokusů, z nichž k vůli podrobnostem tři uvádím.

1. Tři jedinci mající před krmením lesklá tělesa vyčerpaná a dosahující maximálně $2^1/_2$ šestiny průměru jádra krmeni byli 24 hodiny pšeničným škrobem vařeným. Pozorováni po 24 hodinách ukazovali, že jejich tělesa lesklá naplnila se obsahem a dosáhla velikosti maximálně $6^1/_2$ šestiny průměru jádra.

2. Jedinec, který před pokusem krmen byl nabobtnalým škrobem pšeničným, jeví po úplném jeho ztrávení tělesa lesklá, naplněná obsahem a dosahující maximálně velikosti průměru jádra. Jest krmen znovu nabobtnalým škrobem pšeničným 24 hodiny i ukazuje pozorován po 24 hodinách tělesa lesklá zvětšená, dosahující maximálně 10 šestin průměru jádra.

3. Jedinec, mající před pokusem tělesa lesklá vyčerpaná, dosahující maximálně $1^1/_2$ šestiny průměru jádra, krmen byl 24 hodiny vařeným škrobem pšeničným. Pozorován po 24 hodinách ukazoval tělesa lesklá naplněná obsahem a dosahující velikosti 4 šestin průměru jádra. Současně s tímto jedincem krmen byl za stejných ostatních podmínek jiný jedinec syrovým škrobem pšeničným, i jevil pozorován po 24 hodinách tělesa lesklá, naplněná obsahem a dosahující maximálně 3 šestin průměru jádra.

Z posledních dvou pokusů možno též vyvozovati, že nabobtnalý škrob pšeničný neb vařený ztravován jest v těle Pelomyxy za stejnou dobu intensivněji nežli škrob syrový.

Zkoušel jsem též za účelem trávení v těle Pelomyxy vymrzlý škrob pšeničný. Obdržel jsem jej tím způsobem, že vařil jsem škrob syrový s nadbytkem vody, pak zfiltroval a vodu obsahující škrob rozpuštěný ostavil vymrznutí, načež škrob srazil se v podobě více méně vláknitých útvarů, jimiž byli jedinci Pelomyxy krmeni. K vůli jich poznání v těle Pelomyxy zbarvil jsem vymrzlý škrob lakmusem, kurkumou neb kongovou červení. Za tím účelem barvil jsem roztok škrobový před vymrznutím, načež po vymrznutí objevují se sražené částice škrobové slabě fialově zbarvené (lakmusem), neb pěkně citronově (kurkumou) neb červeně (kongovou červení). Takto zbarveným škrobem vymrzlým krmil jsem jedince Pelomyxy 24 hodiny, načež prohlížel jsem je po 24 hodinách a po dalších dobách. Shledal jsem, že tyto částice škrobové obsaženy jsou v těle Pelomyxy bez zřetelných vakuol neb že jsou obklopeny zřetelnými vakuolami, obyčejně všelijak podle nitkovitého tvaru částic škrobových protaženými. Pozoroval jsem dále, že částice tyto po dalším setrvávání v těle Pelomyxy se umenšují, zakulacují neb ztenčují nabývajíce nezřetelného, v plasmatě se ztrácejícího okraje, až konečně nalezl jsem v těle Pelomyxy jen nepatrné jich zbytky.

Že vymrzlý škrob pšeničný v těle Pelomyxy je ztravován, o tom rozhodně nás přesvědčují změny na vyčerpaných tělesech lesklých, jež po nakrmení tímto škrobem naplňují se obsahem a zveličují se. Z pokusů o tom mne přesvědčujících uvádím následující. Tři jedinci, mající lesklá tělesa vyčerpaná s maximální velikostí 2 šestin průměru jádra, krmeni jsou 24 hodiny vymrzlým škrobem pšeničným (zbarveným kongovou červení); prohlíženi po 24 hodinách, ukazují tělesa lesklá naplněná obsahem, dosahující maximálně 4 šestiny průměru jádra.

Při výzkumu zažívání škrobu pšeničného v těle Pelomyxy hleděl jsem též zjistiti, jaká jest při tom reakce, zda neutralní, neb kyselá, neb alkalická. Za tím účelem barvil jsem škrob kongovou červení, lakmusem a kurkumou a takto zbarveným škrobem krmil jsem jedince Pelomyxy, jež jsem pak delší dobu zkoumal, v jednom případě od 13. listopadu (1894) do 6. dubna (1895), průběhem kteréž doby byli jedinci třikráte krmeni. Syrový škrob pšeničný lze někdy zbarviti kongovou červení a tu shledal jsem, že některá takto zbarvená zrna škrobová nalezala se v těle Pelomyxy obklopená vakuolou barvy červené, při čemž na zrnech byly zřetelné stopy působení enzymu. Nabobtnalý škrob pšeničný i vařený lze pěkně barviti kongovou červení. Shledal jsem, že v těle Pelomyxy zrna nabobtnalého škrobu neb částice vařeného škrobu pšeničného obklopeny jsou nejprvé vakuolami červené barvy, později vakuolami barvy nafialovělé a že konečně zbytky obojího škrobu v těle Pelomyxy nabývají barvy nafialovělé.

Škrob nabobtnalý i vařený pšeničný lze dosti trvanlivě barviti lakmusem (užíval jsem při tom lakmusu dialysou čistěného), když po barvení byly podrobeny vyschnutí. Též vymrzlý škrob, jak jsem již uvedl, lze barviti lakmusem. Zkoumal jsem v těle Pelomyxy škrob nabobtnalý a vymrzlý, obojí lakmusem zbarvený, i shledal jsem, že částice obojího škrobu jsou nejprve obklopeny vakuolami barvy fialové, později červené a že částice samy jsou nejprve barvy fialové, později pak (zbytky jich) barvy červené. Zkoumal jsem ještě vymrzlý škrob kurkumou zbarvený a nalezl jsem, že částice toho škrobu zůstávají v těle Pelomyxy stále žlutě zbarveny.

Na základě pozorování tuto uvedených soudím, že při zažívání škrobu pšeničného v těle Pelomyxy jest reakce neutrální a později kyselá.

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Za účelem zažívání v těle Pelomyxy zkoumal jsem vedle škrobu pšeničného ještě jiné druhy škrobu, a sice: bramborový, palmový a rýžový. Prvé dva druhy náležejí ke škrobu s vrstvami excentrickými, a o tom jest známo, hlavně u škrobu bramborového, že dlouho vzdoruje účinkům enzymů diastatických. Na škrobu takovém nevyskytují se působením enzymů takové charakteristické tvary jako na škrobu s vrstvami soustředěnými, jímž jest na př. škrob pšeničný. Proto, když krmil jsem jedince 24 hodiny uvedeným dvojím škrobem, nemohl jsem, prohlížeje škrobová zrna v těle jedinců po 24 hodinách a po řadě dnů, v jednom případě i po několika měsících (týkalo se to škrobu bramborového) nikdy zjistiti na nich bezpečné stopy porušení, svědčící o jich zažívání v těle Pelomyxy. Skrob rýžový rovněž vzdoruje značně účinkům enzymu. Přece však po nakrmení tímto škrobem nalezl jsem, že v těle Pelomyxy zrna tohoto škrobu jsou po delší době porušována způsobem charakteristickým, totiž tvořením se radiárních kanálků a střední, s těmito kanálky spojené dutiny, tedy podobným způsobem jako když na taková zrna působí enzym diastatický.

Že všechny tři druhy škrobu tuto uvedené ztravují se v těle Pelomyxy, o tom rozhodně se přesvědčíme pozorováním změn na vyčerpaných tělesech lesklých, jež po krmení těmito druhy škrobu naplňují se obsahem a zvětšují se. Učinil jsem mezi jinými následující pokus. Vybráno deset jedinců majících lesklá tělesa vyčerpaná a dosahující maximálně 2 šestin průměru jádra. Dva z nich krmeni 24 hodiny škrobem bramborovým, dva škrobem palmovým, pět škrobem rýžovým (a to částečně syrovým, částečně nabobtnalým), jeden pak nekrmen, chován však 24 hodiny za podmínek jinak stejných s ostatními, totiž v krátké nádobce, jež ponořena do velké nádoby vedle krátkých nádobek, v nichž chováni ostatní jedinci se škrobem. Prohlížením těchto jedinců po 24 hodinách shledáno následující: jedinci obsahující škrob bramborový měli lesklá tělesa naplněná obsahem s maximální velikostí 3 šestin průměru jádra, jedinci se škrobem palmovým a se škrobem rýžovým lesklá tělesa též naplněná vesměs obsahem. U prvních pak byla jejich max. velikost 4 šestiny průměru jádra, u druhých 5 šestin průměru jádra.

Jedinec nekrmený měl lesklá tělesa nezměněná. Byv hned po té v téže nádobce krmen škrobem rýžovým (z téhož materiálu, jímž oněch pět jedinců bylo krmeno) 24 hodiny, ukazoval pozorován po 24 hodinách lesklá tělesa naplněná obsahem a zvětšená maximálně na 5 šestin průměru jádra.

Zrna škrobu bramborového i palmového nalezají se v těle Pelomyxy převážnou většinou bez zřetelných vakuol, jsouce těsně plasmatem objata. Rozetřel jsem jednou zrna škrobu bramborového čáštečně na sklíčku a krmil jsem jimi dva jedince 24 hodiny. Pozorováni po 24 hodinách měli jedinci v těle plno zrn škrobových, zrna neporušená byla bez vakuol, kdežto zrna rozestíráním porušená byla většinou ve vakuolách. To lze tím vysvětliti, že u zrn porušených odhalením vnitřních vrstev dána byla větší možnost rozpustnosti nežli u zrn neporušených.

Za účelem výzkumu zažívání zkoušel jsem též modifikaci škrobu bramborového jodem červeně se barvící, již jsem obdržel tím způsobem, že syrový škrob bramborový vařil jsem dvě hodiny ve 2% kyselině sírové, nechal 48 hodin státi, sfiltroval a zbytky škrobové jodem červeně se barvící dobře vodou oplákl. Krmil jsem jimi 24 hodiny čtyři jedince, již měli lesklá tělesa vyčerpaná a s maximální velikostí 2 šestin průměru jádra. Po 24 hodinách tito jedinci prohlíženi měli v těle plno těchto jodem červeně se barvících zbytků škrobových, na mnoze bez vakuol, lesklá tělesa pak byla naplněna obsahem a dosahovala maximální velikosti 5 šestin průměru jádra. Po šesti dnech prohlížel jsem znovu tři jedince a nalezl v těle jejich již méně těchto zbytků škrobových, jodem ještě se červeně barvících, tělesa lesklá nebyla patrněji zvětšena, zůstávajíce v téže velikosti jako po 24hodinném krmení. Z tohoto pokusu nutno tedy souditi, že i červená modifikace škrobová (zde byla ze škrobu bramborového) jest v těle Pelomyxy ztravována.

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Po pokusech se škrobem v příčině zažívání přistoupil jsem k pokusům s cellulosou. Pěstuje Pelomyxu ve velké nádobě, shledal jsem, že jedinci s oblibou se zdržovali na rozkládajícím se papíru filtrovacím a na bavlně, jež do nádoby s Pelomyxami zúmyslně jsem přidal, chtěje tak nápodobiti poměry v tůních na Ladví, v nichž plno cellulosních zbytků se nalézá. Prohlížením těchto jedinců nalezl jsem, že tělo jejich bylo přeplněno vlákny cellulosními, tak že potrava v těle se nacházející vzhledem k nim téměř mizela. Vedle toho měli tito jedinci v sobě plno lesklých těles, naplněných obsahem a obyčejně značně velkých. Pojal jsem doměnku, že tyto poměry lesklých těles jsou v příčinném spojení se stravováním vláken cellulosních v těle Pelomyxy i přistoupil jsem k pokusům.

Krmíme-li jedince Pelomyxy vlákny cellulosními z bavlny chemicky čisté, z papíru filtrovacího, nebo z dřevní cellulosy, pozorujeme-li pak jedince po delší době, nalezneme, že tělo jejich jest přeplněno těmito vlákny, na nichž však není žádných patrnějších stop změny nějaké. Zbarvíme-li taková vlákna (před krmením) kongovou červení, shledáme, že v těle jedinců kolem některých vláken objevují se červené vakuoly, což zřejmě svědčí o tom, že vlákna ta jsou v těle Pelomyxy rozpouštěna. Že jsou i ztravována, o tom přesvědčiti mají pokusy, jež tuto uvádím.

1. Jedinec mající tělesa lesklá úplně vyčerpaná, dosahující maximálně 2 šestin průměru jádra, krmen jest v dlouhé nádobce vlákny cellulosními z bavlny a papíru filtrovacího po 8 dní. Pak prohlížen ukazuje, že tělo jest přeplněno vlákny cellulosními a že tělesa lesklá jsou naplněna obsahem dosahujíce maximálně přes 5 šestin průměru jádra.

2. Jedinec téměř beze zbytků potravy a s tělesy lesklými úplně vyčerpanými, dosahujícími maximálně něco přes 2 šestiny průměru jádra krmen po 4 dny v dlouhé nádobce vlákny cellulosními z chem. čisté bavlny. Prohlížen pak ukazuje, že tělo jest takřka přecpáno vlákny cellulosními a že lesklá tělesa jsou naplněna obsahem, dosahujíce maximálně 5 šestin průměru jádra.

3. Dva jedinci téměř beze zbytků potravy a s tělesy lesklými úplně vyčerpanými, dosahujícími maximálně 2 šestin průměru jádra, krmeni v dlouhé nádobce vlákny cellulosními z chemicky čisté bavlny po 24 hodiny. Pak prohlíženi ukazují, že těla jejich jsou naplněna vlákny cellulosními a že lesklá tělesa jsou naplněna obsahem, dosahujíce maximálně něco přes 3 šestiny průměru jádra.

4. Tři jedinci, mající lesklá tělesa vyčerpaná a dosahující maximálně 2 šestin průměru jádra, krmeni jsou po 24 hodiny vlákny cellulosními z papíru filtrovacího; na to prohlíženi ukazují v těle plno vláken a tělesa lesklá naplněná obsahem a dosahující max. 3 šestin průměru jádra.

5. Jedinec mající tělesa lesklá nevyčerpaná, dosahující max. 3 šestiny průměru jádra, krmen jest vlákny cellulosními z chemicky čisté bavlny 24 hodiny v dlouhé nádobce, aniž tato dána do velké nádoby. Hned po 24 hodinách pozorován, ukazuje plno vláken v těle a tělesa lesklá zvětšená, mající maximálně až přes průměr jádra.

6. Jedinec velký, mající tělesa lesklá vyčerpaná, dosahující maximálně 2 šestin průměru jádra, rozpůlen. Jedna půlka krmena v krátké nádobce, ponořené do velké nádoby, vlákny cellulosními z chem. čisté bavlny. Druhá půlka dána do dlouhé nádobky, jež vložena poblíž krátké nádobky do velké nádoby, kde ponechány obě 24 hodiny. Potom obě prohlíženy. Půlka krmená vlákny cellulosními má plno těchto vláken v těle a tělesa lesklá naplněná obsahem a dosahující maximálně 3 šestin průměru jádra. Půlka nekrmená má v těle lesklá tělesa nezměněná.

7. Dva jedinci, z nichž jeden má tělesa lesklá vyčerpaná, dosahující maximálně 2 šestin průměru jádra, druhý lesklá tělesa částečně jen vyčerpaná, dosahující maximálně 3 šestin průměru jádra, jsou rozpůleni. Jedny půlky krmeny vlákny cellulosními v dlouhé nádobce, vložené do velké nádoby. Druhé půlky dány do jiné nádobky, jež vložena na blízku první do velké nádoby,

kdež obě ponechány po 24 hodiny. Pak oboje půlky prohlíženy. Půlky krmené jeví v těle plno vláken cellulosních, půlka prvního jedince má tělesa lesklá naplněná obsahem a dosahující 3 šestin průměru jádra, půlka druhého jedince má tělesa lesklá naplněná obsahem a dosahující maxim. 5 šestin průměru jádra. Obě nekrmené půlky mají tělesa lesklá nezměněná, jsou však na to v téže dlouhé nádobce krmena po 27 hodin vlákny cellulosními z téhož materiálu, z jakého krmeny první půlky. Po krmení ukazují obě půlky v těle plno vláken a půlka prvního jedince má tělesa lesklá naplněná obsahem a dosahující max. 3 šestin průměru jádra, půlka z druhého jedince má tělesa lesklá naplněná obsahem a dosahující max. 5 šestin průměru jádra.

8. Dva jedinci, mající tělesa lesklá vyčerpaná a dosahující maxim. 2 šestin průměru jádra, krmeni jsou po 24 hodiny vlákny cellulosními z cellulosy dřevní. Na to prohlíženi ukazují v těle plno vláken a tělesa lesklá naplněná obsahem a dosahující max. 3 šestin průměru jádra. Jsou pak dále krmeni týmiž vlákny cellulosními 7 dní. Pak prohlíženi ukazují v těle opět plno vláken a plno lesklých těles dosahujících maximálně 12 šestin průměru jádra.

Z výsledků uvedených tu pokusů nutno vyvozovati, že po krmení cellulosou jednak lesklá tělesa vyčerpaná naplňují se obsahem a zvětšují se, jednak tělesa lesklá již naplněná obsahem se zvětšují, tudíž že cellulosa jest v těle Pelomyxy ztravována.

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Za účelem výzkumů zažívání v těle Pelomyxy pokusil jsem se vpraviti do těla Pelomyxy též ve vodě snadno rozpustné uhlohydráty, zvláště cukry a glykogen. Učinil jsem ve směru tom řadu pokusů, abych mechanicky tyto uhlohydráty do těla Pelomyxy vpravil, užil jsem spodia, blánky kollodiové co obalné blánky a j. Pokusy ty však provázeny byly výsledky neurčitými, pročež upustil jsem od dalšího jich provádění i zdokonalování.

Připadl jsem pak na myšlénku, vpraviti do těla Pelomyxy cukry spojené chemicky s nějakou indifferentní látkou. Za tím účelem uchýlil jsem se ku glykosidům a vybral jsem mezi nimi k pokusům svým především koniferin. Vybral jsem jej předpokládaje, že složka jeho s cukrem (glykosou) spojená, alkohol koniferylový, bude pro tělo Pelomyxy lhostejnou. Jinak zvolil jsem jej i pro menší jeho rozpustnost vzhledem k jiným glykosidům, jež by se byly též hodily k pokusům mým.

Krmení jedinců koniferinem dálo se v úzkých nádobkách. Do nádobek dána voda z velké nádoby, na to ponořen na kulaté dno její jedinec, jenž měl býti krmen, přidány krystalky koniferinu a na to rychle dána vrstva indifferentního humusu, po případě spodia, čímž mělo býti zvolněno rozpouštění koniferinu do vody nad vrstvou a tím spíše umožněno, aby krystalky ještě nerozpuštěné přijaty byly do těla Pelomyxy. Nádobka s obsahem takto upraveným dána do velké nádoby a ponechána tu určitou dobu, načež jedinec vyňat a prohlížen. Provedl jsem pak s jedinci takto opatřenými řadu pokusů, z nichž uvádím následující.

1. Tři jedinci s tělesy lesklými vyčerpanými, které u dvou mají max. velikost 2 šestin průměru jádra, u třetího pak max. velikost $2^1/_2$ šestiny průměru jádra, ponecháni v nádobce s koniferinem 24 hodiny. Pak pozorováni ukazují, že dva nemají v těle žádného koniferinu a lesklá tělesa mají nezměněná, třetí však že má v těle plno krystalků koniferinu buď bez vakuol, buď ve vakuolách, lesklá tělesa jeho že pak jsou naplněna obsahem a dosahují max. velikosti 3 šestin průměru jádra. Na to byl tento jedinec isolován a ponechán bez potravy ve velké nádobě po další dva dny. Potom prohlížen ukazuje, že v těle nalézají se ještě četné krystalky koniferinu vesměs ve vakuolách a se zřetelnými stopami rozpouštění, vedle nich pak četné vakuoly s tekutým obsahem. Dále pak ukazuje, že v těle jsou četná tělesa lesklá, naplněná obsahem a dosahující maximálně 5 šestin průměru jádra. Jedinec tento jest ještě dále isolován bez potravy po dalších 9 dní. Na to prohlížen nemá již v těle žádných stop po krystalcích koniferinu, a lesklá tělesa jeho jeví se umenšena dosahujíce maximálně 4 šestin průměru jádra.

2. Dva jedinci mající lesklá tělesa úplně vyčerpaná a dosahující maximálně 2 šestin průměru jádra ponecháni jsou v nádobce s koniferinem 24 hodiny. Pak prohlíženi ukazují, že tělo jejich jest téměř přecpáno krystalky koniferinu (bez zřetelných vakuol) a že tělesa lesklá jsou naplněna obsahem dosahujíce maximálně 4 šestin průměru jádra. Jedinci pak jsou dále isolováni bez potravy po 2 dny. Potom pozorováni ukazují, že tělesa lesklá jsou naplněna obsahem a mají maximální velikost 5 šestin průměru jádra. Jeden z nich má v těle již málo krystalků koniferinu (ve vakuolách), druhý ještě hojně krystalků, vesměs ve vakuolách.

3. Jedinec veliký, mající lesklá tělesa vyčerpaná a dosahující maxim. $2^1/_2$ šestiny průměru jádra, jest rozpůlen. Jedna půlka nechána v úzké nádobce s koniferinem a humusem 24 hodiny, načež isolována do dlouhé nádobky a ponechána v ní 2 dny bez potravy. Druhá půlka dána do dlouhé nádobky bez koniferinu, pouze s humusem z téhož materiálu jako humus v úzké nádobce. Nádobka s touto půlkou vložena do velké nádoby a ponechána nejprve vedle úzké nádobky s isolovanou první půlkou a s koniferinem 24 hodiny, pak vedle dlouhé nádobky s isolovanou první půlkou 2 dny. Pak obě půlky prohlíženy.

První půlka má v těle jen nepatrné zbytky potravy, za to však krystalky koniferinu, vesměs ve vakuolách, dále vakuoly s tekutým obsahem a jiné s tekutým obsahem a se zrnitými shluky. Tělesa lesklá pak naplněna jsou obsahem a dosahují maximální velikosti 5 šestin průměru jádra. Druhá půlka za isolace rozdělila se na dvě samostatné části; obě však jsou bez potravy, chovají jen něco humusu v těle a jejich lesklá tělesa jsou nezměněna. — (Sr. tab. II., fig. 23.)

4. Jedinec veliký téměř bez stopy potravy a mající tělesa lesklá vyčerpaná a dosahující maximálně velikosti průměru jádra rozdělen jest na 4 části Dvě dány do úzké nádobky s koniferinem a s práškem spodiovým, druhé dvě do jiné úzké nádobky pouze s práškem spodiovým, obě pak nádobky ponechány vedle sebe ve velké nádobě 24 hodiny. Prohlíženy pak ukazují první dvě části, že v těle jejich jest jen něco částeček spodiových, za to ohromné množství krystalků koniferinu vesměs ve vakuolách, kromě toho pak množství těles lesklých, naplněných obsahem a dosahujících maximálně 8 šestin průměru jádra. Druhé dvě části průběhem 24 hodin splynuly v jedno těleso, jež prohlíženo po 24 hodinách ukazuje, že obsahuje něco částeček spodiových a že tělesa lesklá jsou nezměněna.

5. Jedinec veliký má nepatrné zbytky potravy a tělesa lesklá nevyčerpaná, dosahující maximálně více než 2 šestiny průměru jádra. Jest rozpůlen. Jedna půlka dána do úzké nádobky s koniferinem a humusem, druhá do dlouhé nádobky s humusem z téhož materiálu jako v nádobce úzké, obě pak nádobky ponechány ve velké nádobě vedle sebe 24 hodiny. Obě půlky potom prohlíženy.

První má v těle množství krystalků koniferinu (bez zřetelných vakuol) a lesklá tělesa zvětšená, dosahující maximálně 4 šestin průměru jádra. Druhá půlka má v těle něco humusu a potravy z humusu vzaté, tělesa lesklá pak jeví tu maximální velikost skoro 3 šestin průměru jádra. Obě půlky pak isolovány bez potravy do dlouhé nádobky, která ponechána ve velké nádobě dva dny. Potom opět prohlíženy. První půlka má ještě hojně krystalků koniferinu, vesměs ve vakuolách, a tělesa lesklá dosahující maximálně 5 šestin průměru jádra. Druhá půlka má v těle něco humusu a vláken bavlněných a ukazuje tělesa lesklá nezměněná.

6. Veliký jedinec má tělesa lesklá úplně vyčerpaná o dvojí velikosti: jedna mají max. velikost $2^1/_2$ šestiny průměru jádra a druhá, jichž jest méně, mají maximální velikost průměru jádra. Jedinec pak rozpůlen. Jedna půlka dána do nádobky s koniferinem a s humusem, druhá do jiné úzké nádobky s humusem z téhož materiálu jako v nádobce první. Obě nádobky ponechány vedle sebe ve velké nádobě 24 hodiny. Pak obě půlky prohlíženy. První jest téměř přecpána krystalky koniferinu a má tělesa lesklá vesměs naplněná obsahem, jedna dosahují max. velikosti 3 šestin průměru jádra, druhá (jichž jest méně) dosahují maximální velikosti nad průměr jádra. Druhá půlka má v těle jen stopy potravy z humusu vzaté a tělesa lesklá nezměněná. (Sr. tab. II., fig. 24.)

Z výsledků uvedených pokusů vysvítá, že po krmení koniferinem vyčerpaná tělesa lesklá v těle Pelomyxy naplňují se obsahem a zvětšují se a že tělesa lesklá obsahem již naplněná se zvětšují. Z toho nutno dále souditi, že koniferin jest v těle Pelomyxy zažíván, že děje se tu, jak pravděpodobno, působením emulsinu podobného enzymu, rozštěpení koniferinu na alkohol koniferylový pro Pelomyxu indifferentní a na glykosu, jež vstřebávána jest v plasma Pelomyxy a proměňována v glykogen.

B) *Látky bílkovité a glykogen.*

Zkoušel jsem řadu látek bílkovitých, jež vpravoval jsem do těla Pelomyxy, abych vyšetřiti mohl, mají-li nějaký vliv na tvoření se těles lesklých. Uvedu pokusy ty a výsledky jejich podle látek, jež jsem zkoušel.

1. Albumin vaječný, sražený (horkem). Provedl jsem dlouhou řadu pokusů, krmil jsem jedince rozpráškovaným albuminem 24 hodiny i delší dobu, prohlížel je po 24 hodinách i po delší době, nalezl jsem pak, že částice albuminu jsou hojně do těla jedinců přijímány. Tvoří se kolem nich vakuoly, jsou na nich patrné stopy rozpouštění a umenšování, lesklá tělesa však zůstávají při tom nezměněna. Z pokusů, provázených vesměs stejným výsledkem, uvádím následující.

a) Jedinec mající lesklá tělesa vyčerpaná, dosahující max. 3 šestin průměru jádra, krmen albuminem 24 hodiny. Pak prohlížen, jeví v těle plno albuminu, však lesklá tělesa jsou nezměněna.

b) Jedinec velký, mající lesklá tělesa vyčerpaná a dosahující max. 2 šestin průměru jádra, krmen albuminem 48 hodin. Pak prohlížen ukazuje plno albuminu, na mnoze ve vakuolách, lesklá tělesa však jsou u něho nezměněna. Je dále isolován a ponechán bez jiné potravy pět dní. Pak prohlížen ukazuje v těle četné veliké vakuoly se zbytky albuminu, však tělesa lesklá jsou u něho nezměněna.

c) Jedinec mající lesklá tělesa vyčerpaná, dosahující max. 2 šestin průměru jádra, krmen jest albuminem 8 dní. Pak prohlížen ukazuje albumin ve vakuolách, lesklá tělesa však nezměněná. Jest krmen potom 24 hodiny škrobem i má po krmení lesklá tělesa zvětšená, dosahující max. 5 šestin průměru jádra.

d) Dva jedinci krmeni albuminem 24 hodiny a pak isolováni (bez potravy) 24 hodiny. Potom prohlíženi ukazují v těle částice albuminu, však lesklá tělesa jsou u nich nezměněna. Jsou dále isolováni (bez potravy) 10 dní. Pak prohlíženi ukazují v těle částice albuminu ve vakuolách, však lesklá tělesa jsou nezměněna.

2. Globulin krystallovaný (ze závodu Grüblerova). Pokus s globulinem tímto ukazuje stejný výsledek jako pokusy s albuminem. Uvádím jej tuto. Pět jedinců, z nichž dva velicí a tři malí, mají lesklá tělesa vyčerpaná, dosahující maximálně jednak 2, jednak 3 šestin průměru jádra. Krmeni 24 hodiny globulinem a pak prohlíženi. Ukazují, že průběhem 24 hodin dva velcí se spojili, všichni pak mají v těle plno krystalků globulinu, však lesklá tělesa jsou nezměněna. Jsou dále isolováni s trochou humusu 4 dny. Pak prohlíženi ukazují v těle plno krystalků globulinu, jinak téměř žádnou potravu; lesklá tělesa jsou u nich nezměněna. Jsou dále isolováni 10 dnů. Pak tři z nich prohlíženi, i ukazují v těle plno krystalků globulinu, vesměs ve vakuolách a s patrnými stopami

rozpouštění, jevícími se na otupených hranách a vyžraných plochách. Tělesa lesklá zůstávají při tom nezměněna.

3. Fibrin. Pokus s fibrinem jeví stejný výsledek jako s albuminem a s globulinem. Uvádím jej tuto. Čtyři jedinci mající lesklá tělesa vyčerpaná, dosahující $1^1/_2$ šestiny průměru jádra, krmeni jsou 24 hodiny rozpráškovaným fibrinem. Pak prohlíženi ukazují v těle hodně částic fibrinových, částečně ve vakuolách, lesklá tělesa jsou však u nich nezměněna. Jsou dále isolováni 5 dní, načež jeden z nich prohlížen jeví v těle částečky fibrinové, však lesklá tělesa zůstávají nezměněna. Jedinci jsou dále isolováni 7 dní. Pak jeden z nich prohlížen ukazuje v těle již málo částeček fibrinových a lesklá tělesa nezměněná.

4. Kasein. Pokusy s kaseinem mají týž výsledek jako pokusy s uvedenými již látkami bílkovitými. Uvedu z nich tento pokus. Několik jedinců majících lesklá tělesa vyčerpaná a dosahujících $1^1/_2$ šestiny průměru jádra krmeno jest kaseinem 24 hodiny. Pak někteří prohlíženi ukazují v těle četné částečky kaseinu, vesměs ve vakuolách, však lesklá tělesa jsou u nich nezměněna. Potom krmeni jsou jedinci dále kaseinem 4 dny, načež prohlíženi jsou dva jedinci; mají v těle částečky kaseinové ve vakuolách, však lesklá tělesa jsou nezměněna. Jeden z těchto dvou jedinců krmen jest 24 hodiny škrobem i ukazuje po krmení lesklá tělesa naplněná obsahem a dosahující maximálně 5 šestin průměru jádra. Ostatní jedinci krmeni dále kaseinem 7 dní. Pak jeden z nich prohlížen má v těle málo částeček kaseinových ve vakuolách a lesklá tělesa nezměněná. Jedinci dále jsou ještě krmeni kaseinem 5 dní. Jeden z nich pak prohlížen, i ukazuje v těle částečky kaseinu (ve vakuolách), lesklá tělesa jsou však u něho nezměněna.

5. Nuclein (ze závodu Grüblerova). Pokusy s nucleinem mají rovněž týž výsledek jako předešlé. Uvedu z nich následující. Tři jedinci mající lesklá tělesa vyčerpaná a dosahující 2 šestin průměru jádra krmeni jsou 24 hodiny nucleinem. Na to prohlíženi ukazují v těle plno nucleinových zrnek a větších částic, však lesklá tělesa jsou nezměněna. Jedinci pak krmeni jsou dále nucleinem 8 dní. Pak jeden prohlížen ukazuje v těle plno zrnek nucleinových a větší částice nucleinové částečně ve vakuolách; lesklá tělesa jsou u něho nezměněna.

6. Gelatina. Gelatina rozpuštěna v horké vodě a sražena kys. tříslovou. Částečkami těmito krmeni jsou 24 hodiny tři jedinci, z nichž dva větší mají tělesa lesklá plná, dosahující maximálně 3 šestin průměru jádra, třetí (menší) má tělesa lesklá dosahující max. 4 šestin průměru jádra. Po krmení prohlíženi ukazují jedinci částečky gelatiny skoro vesměs bez vakuol; lesklá tělesa jsou u nich nezměněna. Jsou dále isolováni s humusem 4 dny. Pak prohlíženi ukazují dva částečky gelatiny načervenalé, většinou ve vakuolách, a lesklá tělesa nezměněná, třetí má částečky gelatinové ve vakuolách a plno humusu, tak že nelze s jistotou určiti poměry lesklých těles. Potom jedinci dále isolováni 6 dní, načež

dva prohlíženi ukazují v těle částečky gelatinové vesměs ve vakuolách a lesklá tělesa nezměněna. Ještě dále jsou isolováni s humusem 16 dní, načež nalezen jen jeden jedinec, jenž má v těle již jen malý počet gelatinových částeček na mnoze velmi umenšených a vesměs ve vakuolách; lesklá tělesa jsou u něho nezměněna. Tento jedinec pak dále isolován s humusem, v němž byla obsažena různá potrava. Prohlížen po 15 dnech ukazuje, že v těle není žádných stop po gelatinových částicích, za to však plno různé potravy, jeho lesklá tělesa jsou zvětšena, dosahujíce maximálně velikosti průměru jádra. Jedinec tento krmen ještě 24 hodiny škrobem pšeničným i ukazuje po krmení tělesa lesklá zvětšená, dosahující maximálně 8 šestin průměru jádra.

* * *

Uvedené pokusy s látkami bílkovitými ukazují, že krmí-li se jimi 24 hodiny neb i delší dobu, lesklá tělesa v těle Pelomyxy se nemění, zvláště že nenaplňují se obsahem a nezvětšují se. Z pokusů nelze ještě dovozovati, že po krmení látkami bílkovitými netvoří se glykogen v těle Pelomyxy, nýbrž jen tolik lze tvrditi, že po krmení látkami bílkovitými trvajícím 24 hodiny i delší dobu nehromadí se glykogen ve vyčerpaných tělesech lesklých.

* * *

Když jsem zjistil, že albumin sražený horkem nezpůsobuje v těle Pelomyxy po 24 hodinách i po delší době hromadění se obsahu ve vyčerpaných tělesech lesklých, pokusil jsem se vpraviti do těla Pelomyxy glykogen obsažený ve sraženém albuminu, abych mohl vyšetřiti, nastanou-li v tomto případě změny na vyčerpaných tělesech lesklých. V bílku vaječném rozpustil jsem velké množství glykogenu, pak jsem bílek horkem srazil, nechal vyschnouti a rozpráškoval. Takto upraveného bílku užil jsem k následujícím dvěma pokusům.

1. Velký jedinec mající tělesa lesklá vyčerpaná a dosahující maximálně něco přes průměr jádra jest rozpůlen. První půlka krmena v úzké nádobce bílkem s glykogenem, druhá půlka v jiné úzké nádobce bílkem bez glykogenu; obě nádobky ponechány 24 hodiny vedle sebe v nádobě velké, načež obě půlky jsou prohlíženy. I ukazuje první půlka, že v těle jest plno částeček bílku, lesklá tělesa jsou pak naplněna obsahem a zvětšena dosahujíce maximálně dvojnásobného průměru jádra. Druhá půlka jeví v těle rovněž plno částeček bílku, však tělesa lesklá jsou nezměněna. (Sr. tab. II., fig. 25.)

2. Jedinec, jenž měl plná a velká tělesa lesklá, byl delší dobu isolován za nedostatku potravy, až ukazuje tělesa lesklá úplně vyčerpaná, dosahující maximálně něco přes průměr jádra. Na to jest rozpůlen. První

půlka krmena jest v úzké nádobce bílkem s glykogenem, druhá půlka v jiné úzké nádobce bílkem bez glykogenu, však pocházejícím z téhož materiálu jako bílek s glykogenem. Obě nádobky ponechány pak vedle sebe 24 hodiny ve velké nádobě, načež obě půlky prohlíženy. První půlka téměř přeplněna jest částečkami bílku, ukazujíc tělesa lesklá naplněná obsahem a zvětšená maximálně na 8 šestin průměru jádra. Jodem ukazují tělesa lesklá totéž zbarvení jako glykogen, jenž bílkem byl do těla vpraven. Druhá půlka ukazuje v těle rovněž plno částeček bílku, však tělesa lesklá jsou tu nezměněna.

* * *

Z výsledku obou pokusů musíme souditi, že glykogen s bílkem v tělo Pelomyxy vpravený jest zažíván a ukládán v podobě obsahu do vyčerpaných těles lesklých.

C) *Tuky.*

Učinil jsem řadu pokusů, jimiž snažil jsem se zjistiti chování se tuku v těle Pelomyxy vzhledem k tělesům lesklým. Pokusy ty náležejí k pokusům velmi obtížným, poněvadž tukové kapky vpravují se do těla Pelomyxy v dostatečném množství velmi nesnadno.

Po zkouškách s různými tuky užíval jsem k rozhodujícím pokusům tuku v mléce obsaženého a tuku rybího. Z tuku rybího učinil jsem emulsi v bílku vaječném, tento srazil horkem, nechal pak vyschnouti a rozstrouhal na prášek, jímž krmil jsem pokusné jedince. Pokusy s tukem mléčným prováděl jsem tímto způsobem: Do nádobek pokusných, naplněných vodou, dal jsem jedince, k nim na dno opatrně vpravil pipetou několik kapek mléka, načež rychle jsem přidal k tomu něco humusu (neobsahujícího žádné téměř potravy), čímž docílil jsem, že kapky tukové byly na mnoze humusem zadrženy a tím umožněno, že mohly býti snadněji (s humusem) přijaty do těla Pelomyxy. Z pokusů uvádím následující:

1. Tři jedinci mající lesklá tělesa vyčerpaná, dosahující maximálně 2 šestin průměru jádra, krmeni jsou v úzké nádobce tukem mléčným 24 hodiny, načež prohlíženi ukazují, že v těle jest plno humusu a kapek tukových, však lesklá tělesa v nich jsou nezměněna.

2. Ze šesti jedinců s tělesy vyčerpanými dosahujícími max. 2 šestin průměru jádra, krmeni jsou tukem mléčným tři v úzké nádobce a tři v dlouhé nádobce 24 hodiny, načež prohlíženi ukazují, že v těle jejich jest plno humusu a kapek tukových, že však lesklá tělesa jejich jsou nezměněna. Tři z nich dále byli isolováni, i shledáno při dalším prohlížení, že vypustili z těla humus a podrželi v něm jen četné kapky tukové. Desátého dne isolace jsou znovu prohlíženi, i mají v těle jen málo kapek tukových; lesklá tělesa jsou nejen vyčerpána, nýbrž u dvou jedinců jsou spečena ve skupiny (agglutinována). Pak byli jedinci opětně krmeni

24 hodiny tukem mléčným. Po krmení ukazovali v těle mnoho humusu a kapek tukových, však lesklá tělesa byla v nich nezměněna. Jedinci dále isolováni po čtyry dny, při čemž jeden z nich zahynul. Ostatní dva prohlíženi čtvrtého dne neukazují v těle již žádné stopy po kapkách tukových; jsou pak 24 hodiny krmeni tukem mléčným. Při krmení jeden zahynul, druhý po krmení prohlížen ukazuje v těle málo kapek tukových a má tělesa lesklá nezměněna.

3. Tři jedinci, mající lesklá tělesa vyčerpaná, dosahující maximálně $2^1/_2$ šestiny průměru jádra, krmeni jsou 24 hodiny tukem rybím ve sraženém bílku. Prohlíženi pak ukazují, že v těle jest plno částeček bílkových s kapkami tukovými a též samotné kapky tukové, lesklá tělesa však jsou u nich nezměněna. Jsou isolováni přes den a ukazují potom v těle hodně částeček bílkových s kapkami tukovými i samotné kapky tukové, obojí na mnoze ve vakuolách; tělesa lesklá jsou však u nich nezměněna. Pak jsou jedinci dále ještě krmeni 8 dní. Potom prohlíženi ukazují dva v těle hojně, třetí méně hojně částeček bílkových s kapkami tukovými a samotné kapky tukové; lesklá tělesa jsou u nich nezměněna.

⁂

Pokusy tyto svědčí o tom, že po krmení 24hodinném i delší dobu trvajícím tukem mléčným neb rybím neděje se v těle Pelomyxy hromadění obsahu (glykogenu) ve vyčerpaných tělesech lesklých.

Závěrek.

Zjistil jsem, že tělesa lesklá v těle Pelomyxy se nalézající skládají se z obsahu, jenž jest glykogenem, a z blány obalné, jež jest těžko rozpustným uhlohydrátem. Zjistil jsem též, že glykogen hromadí se v tělesech lesklých po hojném krmení potravou uhlohydrátovou neb uhlohydrát obsahující (škrobem, jeho různými modifikacemi, glykogenem, cellulosou a koniferinem). Konečně zjistil jsem, že glykogen jsa látkou živnou, v níž uložena jest energie potenciálná, pro děje životní upotřebitelná, jest za nedostatku potravy z těles lesklých vyčerpáván až do úplného vymizení a že toto ubývání glykogenu provázeno jest ubýváním děje životního pohybem vyjádřeného.

Myslím, že glykogen tvoří se v těle Pelomyxy, zvláště aspoň tehdy, když hromadí se v leských tělesech, přímo proměnou z potravy uhlohydrátové neb uhlohydráty obsahující a nikoliv odštěpováním z plasmatu, i myslím, že podporou domněnky této jsou pozorování a pokusy mé v této práci uvedené.

Ze způsobu tvoření se lesklých těles možno vyložiti též odchylné tvary těles lesklých. Tak tvary piškotovité povstávají asi částečným splynutím dvou na blízku povstalých těles, tvary nepravidelné asi splynutím částečným většího počtu na blízku povstalých těles. Na základě pozorování, že v plasmatě

Pelomyxy objevují se časem lesklá tělesa s blanou protrženou a jinak porušenou, soudím, že blána obalná nezůstává trvale v činnosti a že po delší době činnosti své podrobena jest zkáze, čímž jest i dána zkáza tělesa, k němuž náleží. Soudím pak dále, že takto zaniklá tělesa nahrazována jsou novými. Myslím, že při hojné potravě děje se stále tvoření nových těles. Svědčí o tom úkaz, že při hojném krmení jedinců Pelomyxy nalézal jsem v těle jejich vedle naplněných starých lesklých těles tělesa lesklá nejmenší velikosti, jichž přítomnost nemohla se před krmením zjistiti, o nichž musím tudíž souditi, že se utvořila teprve průběhem zažívání.

R. Greeff, zasloužilý badatel v oboru prvoků, jenž Pelomyxu objevil a důkladně popsal,*) považuje lesklá tělesa za spory Pelomyxy i domnívá se o nich, že pocházejí ze zvláštních těles v jádře Pelomyxy se vyskytujících. Tato tělesa, kulovitá, lesklá a plná, mění se za zvětšování objemu jádra v tělesa s dvojitou konturou, kteráž protržením blány jaderné ven do plasmatu se dostávají a zde lesklými tělesy se stávají. Domněnku Greeffovu o významu lesklých těles vyvrací tuto uvedená pozorování má i pokusy. mé. Také jeho domněnku o původu těles lesklých jest mi považovati za mylnou. Jak Greeff ukázal, vyskytují se v jádře zvláštní tělesa kulovitá, bledě lesklá, nezbarvená a neprůhledná. Velikosti jsou různé, Bývají nezřetelná a nalézají se v jádře ve větším počtu, neb jsou patrna, značnější velikosti a v menším počtu, konečně — to pozoroval jsem u jedinců vyhladovělých — dosahují i extremní velikosti až $2\frac{1}{2}$ šestiny průměru jádra a počet jich v jádře může býti umenšen až na jedno.

V kyselině octové, jak již Greeff ukázal, jsou tělesa tato rozpustna, rovněž v kys. solné a dusičné. Myslím, že tělesa s dvojitou konturou Greeffem pozorovaná a uváděná v souvislost s popsanými právě tělesy, byla vyčerpaná tělesa lesklá menších rozměrů a že pozorování jeho spočívalo na omylu, jenž vznikl tím, že vyčerpaná lesklá tělesa s jádrem agglutinovaná vykládal, jako by se nalézala uvnitř jádra. Že tělesa v jádře Pelomyxy se nalézající různí se od těles lesklých, zvláště od vyčerpaných těles lesklých, o tom svědčí již různé chování se obou k chemickým činidlům. Myslím, že uvedená tělesa jsou produktem děje chemického v jádře se dějícího a domnívám se o nich, že jsou povahy tukové, mám však o tom ještě některé pochybnosti, i hodlám se vrátiti k důkladnějšímu jejich studiu v práci pozdější.

Jest dosud málo prací, které zabývaly se otázkou zažívání uhlohydrátů u amoeb, ty pak týkají se pouze zažívání škrobu syrového. Greenwood**) a Meissner***) dospěli při tom k výsledku zápornému.

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*) *Pelomyxa palustris, ein amoebenartiger Organismus des süssen Wassers.* (Archiv für mikroskopische Anatomie, X. Bd., 1874.)

**) *On the digestive process in some Rhizopods* (Journal of Physiology, VII., 1886).

***) *Beiträge zur Ernährungsphysiologie der Protozoen* (Zeitschrift für wissenschaftliche Zoologie, 46 Bd., 1888).

Naproti tomu Hartog a Dixon*) dobyli z těla Pelomyxy vedle pepsinu enzym diastatický, což jest ve shodě s výsledky mých pozorování a pokusů.

Meissner činil též pokus s Pelomyxou krmiv ji 24 hodiny škrobem rýžovým i nalezl, že po těchto 24 hodinách škrob se nezměnil. Domnívám se, že příčinu dosavadních záporných výsledků hledati sluší v nedostatečnosti methody zkoumací a též v tom, že nebyl vzat zřetel na různé chování se různých druhů škrobu syrového vzhledem k enzymům diastatickým.

Myslím, že u amoeb a sarkodin vůbec děje se zažívání a tvoření se uhlohydrátů, že však nemůže se tak patrně zjistiti jako u Pelomyxy. Tak na př. nalezl jsem u Amoeby proteus, kterou jsem pěstoval za podmínek, pokud možno, přirozených, že pohlcovala, ač v malém množství, zrna syrového škrobu pšeničného a že je porušovala způsobem charakteristickým pro enzym diastatický.

Zjištěním změn na přijaté potravě není ještě podán přesný důkaz o jejím zažívání. V případě škrobových zrn s vrstvami excentrickými vidíme, že jsou zažívána, aniž přesně lze zjistiti změny na zrnech. Přesný důkaz zažívání látky nějaké podán je tehdy, podaří-li se nám zjistiti příčinnou souvislost změn na této látce pozorovaných se změnami tělesa, jež jest součástkou plasmatu, v němž látka jest proměňována. To, myslím, podařilo se mi u Pelomyxy, totiž zjištěním chemické povahy lesklých těles a zjištěním příčinné souvislosti změn na nich pozorovaných se změnami přijaté potravy.

Domnívám se, že otevřel jsem tu jednu cestu k podrobnějšímu zbadání dějů životních u nejnižších ústrojenců živočišných. Doufám, že methoda pěstovací, pozorovací i pokusná, jichž jsem u Pelomyxy užil a zde je též popsal, umožní badatelům pozorování a pokusy mé kontrolovati.

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*) *On the Digestive Ferments of a large Protozoon* (Rep. 63. Meet. Brit. Ass. Ad. Sc., dle referátu v Zoologischer Jahresbericht, 1895).

Vysvětlení tabulek.

Tabulka I.

Fig. 1. Trávené zrno syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 24 hodinách (od počátku krmení). Kresleno při zvětš. 350.

Fig. 2. Trávené zrno syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 2 dnech (od počátku krmení) Kresleno při zvětšení 350.

Fig. 3. Trávená zrna syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 3 dnech (od počátku krmení). Kresleno při zvětšení 350.

Fig. 4. Trávená zrna syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 5 dnech (od počátku krmení). Kresleno při zvětšení 350.

Fig. 5. Trávená zrna syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 6 dnech (od počátku krmení). Kresleno při zvětšení 350.

Fig. 6. Trávená zrna syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 7 dnech (od počátku krmení). Kresleno při zvětšení 350.

Fig. 7. Trávená zrna syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 8 dnech (od počátku krmení). Kresleno při zvětšení 350.

Fig. 8. Trávená zrna syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 9 dnech (od počátku krmení). Kresleno při zvětšení 350.

Fig. 9. Zrna syrového škrobu pšen., jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 12 dnech (od poč. krmení). *a* až *k* zrna trávená; *l*, *m*, zrna neporušená. Kresl. při zvětšení 350.

Fig. 10. Trávená zrna syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 13 dnech (od počátku krmení). Kresleno při zvětš. 350.

Fig. 11. *A.* Trávená zrna syr. škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hod. a jenž prohlížen po 6 dnech (od poč. krmení). I., II. zrna ve vakuolách potravních. Kresl. při zvětš. 350.

Fig. 11. *B.* Trávená zrna syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po 14 dnech (od počátku krmení). Kresleno při zvětš. 350

Fig. 12. Trávená zrna syr. škrobu pšen. (částečně ve vakuolách potr.), jímž krmen jedinec Pelomyxy 1 hodinu a jenž prohlížen po 44 hodinách (od počátku krmení). Kresl. při zvětš. 350.

Fig. 13. *A.* Trávená zrna syr. škrobu přeničného (v potravních vakuolách), jímž krmen jedinec Pelomyxy $^1/_2$ hod. a jenž prohlížen po 24 hodinách (od počátku krmení). Kresleno při zvětš. 350.

Fig. 13. *B* a 13 *C.* Trávená zrna syrového škrobu pšeničného (v potravních vakuolách až na *Cg*), jímž krmen jedinec Pelomyxy $^1/_2$ hod. a jenž prohlížen po 2 dnech a pak po 4 dnech (od počátku krmení). Kresleno při zvětš. 350.

Fig. 14. Trávená zrna syrového škrobu pšen. (částečně v potravních vakuolách), jímž krmen jedinec Pelomyxy $^1/_2$ hod. a jenž prohlížen po 24 hodinách (od počátku krmení). Kresl. při zvětš. 350.

Tabulka II.

Fig. 15. *A* a 15.' *B.* Trávená zrna syr. škrobu pšen. (částečně v potravních vakuolách), jímž krmen jedinec Pelomyxy 24 hodiny a jenž prohlížen po $4^1/_2$ hodinách a pak po 29 hodinách (od počátku krmení). Kresleno při zvětš. 350.

Fig. 16. Trávená zrna syrového škrobu pšeničného, jímž krmen jedinec Pelomyxy 4 hodiny a jenž prohlížen po 6 dnech (od počátku krmení). Kresleno při zvětš. 350.

Fig. 17. Lesklá tělesa: *A* před, *B* po 1hodinném setrvání v destillované vodě. Zvětšeno asi 140.

Fig. 18. Lesklá tělesa po působení sekretu žlaz slinných. *A* po působení 24hodinném. *B* po působení 4 dny trvajícím (nahoře lesklá tělesa, na něž působil sekret s glycerinem a destillovanou vodou, dole lesklá tělesa, na něž působil pouhý sekret). Zvětšeno asi 140.

Fig. 18 * *a* lesklé těleso (dvojité) před působením glycerinového výtažku pankreasu hovězího (s chloroformem); *b* totéž těleso po 4hodinném působení téhož výtažku V této podobě zůstává i po dalším působení téhož, však obnoveného výtažku Zvětš. asi 140.

Fig. 19. Jedinec Pelomyxy krmen 24 hodiny syrovým škrobem pšeničným. *A* lesklá tělesa jeho (většinou agglutinovaná) před krmením; *B* tatáž po krmení; *j* jádro s agglutinovanými (vyčerpanými) tělesy lesklými, jež nedopatřením vyobrazena jakoby plná byla. Zvětš. asi 300.

Fig. 20. Jedinec Pelomyxy isolován a ponechán bez potravy 6 dní. (Má na začátku isolace lesklá tělesa plná, dosahující max. průměru jádra.) Týž krmen pak 24 hodiny škrobem pšen. syrovým. *a* až *g* lesklá tělesa před krmením; *A* až *E* lesklá tělesa po krmení; *j* jádro (s bakteriemi). Zvětšení asi 600.

Fig. 21. Jedinec Pelomyxy krmen 48 hodin škrobem pšen. syrovým. *A* lesklá tělesa před krmením (agglutinovaná); *B* táž po krmení (částečně ještě agglutinovaná); j_1 až j_3 jádra (částečně agglutinovaná) před krmením; J_1 až J_3 táž po krmení (částečně agglutinovaná). Zvětš. asi 300.

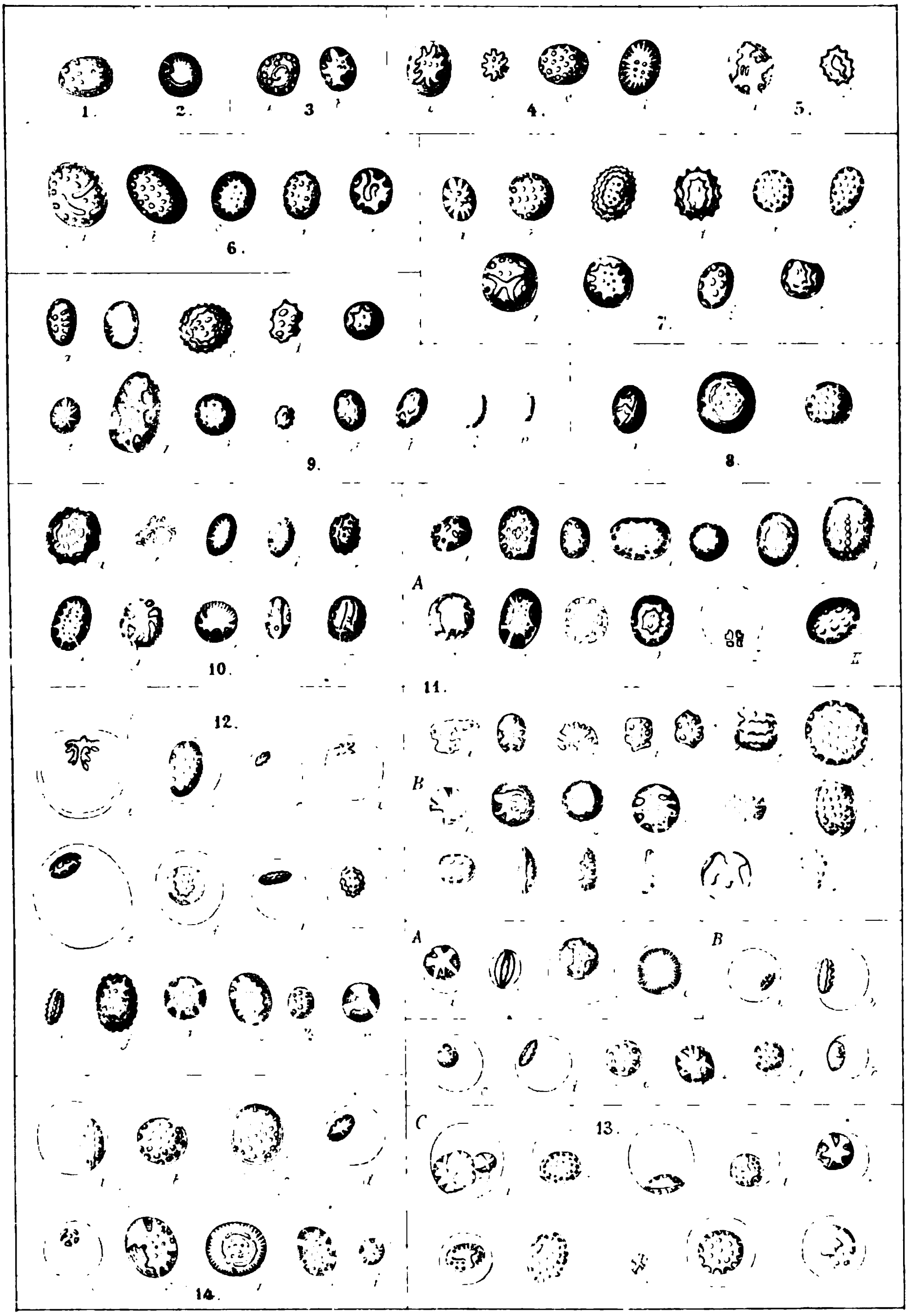
Fig. 22. Jedinec Pelomyxy jest isolován bez potravy 13 dní, pak krmen 24 hodiny škrobem pšeničným syrovým. B_1 lesklá tělesa na počátku isolování. *A* táž na konci isolování (před krmením); B_2 táž po krmení; j_1 a j_2 jádra (agglutinovaná) na konci isolování (před krmením). Zvětšeno asi 250.

Fig. 23. Půlka jedince Pelomyxy krmena 24 hodiny krystalky koniferinu a prohlížena po 48 hodinách (od konce krmení). *A* lesklá tělesa před krmením; *B* táž po krmení; j_1 a j_2 jádra. Zvětšení asi 300.

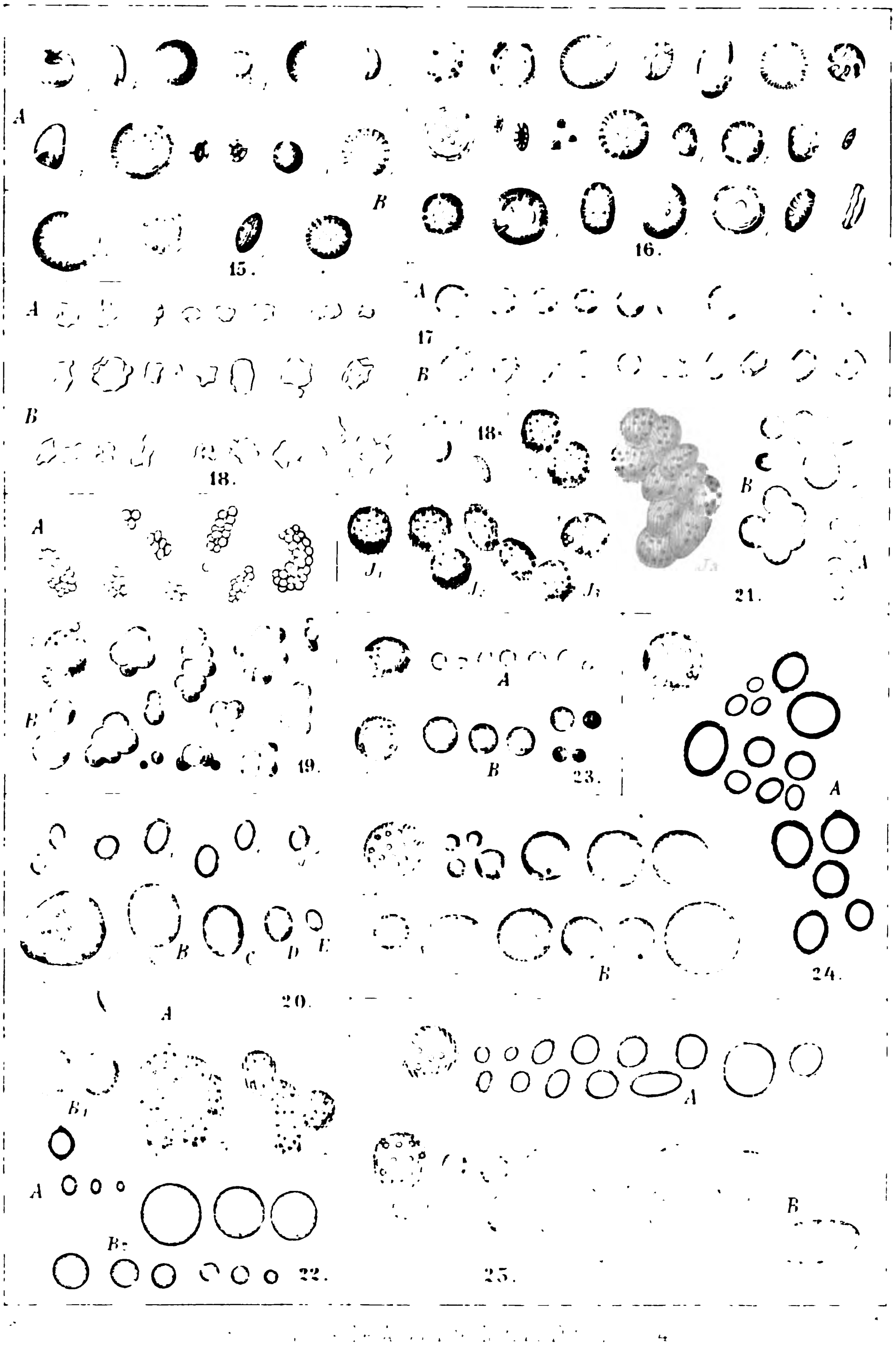
Fig. 24. Půlka jedince Pelomyxy krmena 24 hod. krystalky koniferinu a prohlížena po 24 hodinách (od počátku krmení). *A* lesklá tělesa před krmením; *B* táž po krmení; j_1 a j_2 jádra. Zvětšení asi 400.

Fig. 25. Půlka jedince Pelomyxy krmena 24 hodiny bílkem sraženým s glykogenem a prohlížena po 24 hodinách (od počátku krmení). *A* lesklá tělesa před krmením; *B* táž po krmení; j_1 a j_2 jádra. Zvětšení skoro 400.

Tab I.



Tab. II.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Plasmodiogonie,

ine Vermehrungsart der niedersten Protozoen

3

Nach den Untersuchungen an mehrkernigen Formen
der *Amoeba proteus*

von

Antonín Štolc

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Prof. Dr. Wilh. Roux,
Halle a/d. S. (Deutschland).

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Plasmodiogonie, eine Vermehrungsart der niedersten Protozoen.

Nach den Untersuchungen an mehrkernigen Formen der *Amoeba proteus*.

Von

Antonín Štolc.

Eingegangen am 14. Dezember 1905.

In dieser Arbeit wird der Versuch gemacht, die Bedeutung der mehrkernigen Formen im Lebenscyclus von *Amoeba proteus* zu erklären. Hierzu dienten mir teils einige Ergebnisse meiner früheren Arbeit[1]), teils Resultate andrer Untersuchungen, bei welchen Amöben auf die in meiner zitierten Arbeit angegebene Weise kultiviert wurden.

Bei der Lösung der Frage nach der Bedeutung mehrkerniger Formen schien es mir wichtig zu sein, vorerst zu untersuchen, unter welchen Umständen und wie überhaupt diese Formen entstehen, was für Veränderungen im Vergleich mit den einkernigen Formen die Mehrkernigkeit zur Folge hat, und schließlich, wie sich die mehrkernigen Formen fernerhin verhalten, besonders dann, wenn sie Teilungen eingehen.

I.

A. Die mehrkernigen Formen können bei Nahrungsmangel entstehen.

Hierfür gibt folgende Untersuchung Belege. Ein einkerniges Individuum wurde im Uhrglas unter täglichem Ersatz an frischem

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[1]) Über die Teilung des Protoplasmas im mehrkernigen Zustande. Nach den Untersuchungen an merkernigen Formen der *Amoeba proteus*. Archiv f. Entw.-Mech. Bd. XIX. 1905.

Wasser nebst neuer Nahrung kultiviert. Es erfolgte bald eine Zweiteilung, deren Produkte einkernig waren — eine Erscheinung, welche sich auch an dem einen Tochterindividuum, das allein weiter in Kultur genommen wurde, bei seiner Teilung wiederholte. Ein andres Resultat erhielt ich jedoch, als die Bedingungen von jetzt an geändert wurden. Ich überführte nämlich das eine von den beiden letzten Teilungsprodukten in ein mit frischem Flußwasser gefülltes Cylindergläschen und beließ die Kultur sich selbst, ohne Nahrung hinzuzufügen. Nach 4 Tagen (am 3. Juli 1898) erschien hierauf das Individuum zweikernig. Bei seiner Rückkehr in die oben angeführten Bedingungen (Uhrglas, Wasser- und Nahrungsaustausch) erfuhr es nach der Beobachtung am dritten Tage eine Dreiteilung, deren Produkte aus einem großen und zwei kleinen Individuen bestanden. Ersteres enthielt zwei grobkörnige Nuclei, die beiden letzteren je einen fein granulierten. Fortgesetzte Kultur des großen Individuums unter denselben Bedingungen ergab am zweiten Tage eine stattgefundene Vierteilung, deren Produkte je einen feinkörnigen Nucleus besaßen. Hiermit kehrten die Erscheinungen wieder in ihr altes Geleis zurück, denn als noch zwei der letzten vier Descendenten der Alleinkultur unterworfen wurden, traten wieder dieselben einfachen Verhältnisse, nämlich Zweiteilung, Einkernigkeit der Produkte, zutage, gerade wie am Anfange des Versuchs. Damit wurde schließlich die Untersuchung beendigt.

B. Die mehrkernigen Formen können auch bei Überfluß an Nahrung entstehen.

Einen Beleg hierfür bietet folgende Beobachtung. Vier einkernige Individuen, einer nahrungsarmen Kultur (am 8. Mai 1897) entnommen, wurden unter täglichem Ersatz an frischem Wasser und nach Hinzugabe reichlicher Nahrung in einem Hohlgläschen (ausgehöhltem Objektträger) kultiviert. Die Nahrung bestand aus Infusorien der Gattungen *Paramecium*, *Colpidium* u. a. Am 12. Mai fand ich alle vier Individuen jedes in zwei einkernige Produkte geteilt. Von den neuen Individuen wurden dann nur vier Exemplare allein gewählt und für die Kultur verwendet. Am 18. Mai ließ sich wahrnehmen, daß drei von ihnen in je zwei einkernige Nachkommen sich geteilt haben, das vierte jedoch ungeteilt geblieben ist. Letzteres nahm ich dann für sich allein in Kultur und beobachtete es häufig. Um 3 Uhr nachmittag erschien noch sein Nucleus ungeteilt, bis schließlich um 12 Uhr nachts die Teilung direkt unter dem Mikroskop sich wahrnehmen ließ. Derselben entsprangen zwei einkernige und ein zweikerniges Individuum, alle durch feine Granulierung der Kerne ausgezeichnet. Das zweikernige Produkt wurde dann eingehend weiter beobachtet und zwar einmal nach 2 Uhr nachts (am 19. Mai), dann um 7 Uhr morgens und nachmittag um 1 Uhr, ohne daß eine Teilung auch nur angedeutet wäre. Vorübergehend überführte ich hierauf das Versuchsobjekt in ein Cylindergläschen mit frischem Flußwasser (ohne Nahrung), um es nach 24 Stunden wieder in das betreffende Hohlglas mit frischem Wasser und reichlicher Nahrung zurückzuversetzen. Die Untersuchung am folgenden Tage ergab eine stattgefundene Vierteilung, deren Produkte durchwegs als einkernig sich erwiesen. Bei den hierauf folgenden Teilungen bis zum vierten Gliede, wobei allerdings stets nur ein Individuum in Kultur genommen wurde, traten nur Zweiteilungen und Einkernigkeit zutage. Die betreffenden successiven Teilungen wurden am 22., 24. und 25. Mai konstatiert, worauf von weiteren Beobachtungen Abstand genommen wurde.

C. Die mehrkernigen Formen können in älteren Kulturen auftreten.

Darüber habe ich schon in meiner oben zitierten Arbeit berichtet. Die Amöben wurden in kleinen Cylindergefäßen mit Flußwasser nach Hinzugabe von Infusoriennahrung gezüchtet. Täglich oder nach längeren Zeitabschnitten wurde frisches Wasser und neue Nahrung zugesetzt. In der Regel teilten sich die Amöben eifrig, wobei anfangs nur einkernige, in älteren Kulturen jedoch neben diesen auch mehrkernige, meist zweikernige, minder häufig dreikernige oder vierkernige Individuen auftraten.

D. Die mehrkernigen Formen können auch bei ungünstiger Jahreszeit auftreten.

Durch eine auf 1 Jahr und mehr als 8 Monate ausgedehnte Untersuchung, wobei unter den unten angegebenen Umständen täglich Beobachtungen an *Amoeba proteus* ausgeführt wurden, stellte sich heraus, daß mit Ausnahme des Herbstes, wo mehrkernige (zweikernige) Individuen neben einkernigen auftraten, sonst konstant nur einkernige Formen aus den Teilungen hervorgingen. Die betreffenden Beobachtungen dauerten vom 21. Dezember 1896 bis zum 24. August 1898, wo die beabsichtigte Fortsetzung der Beobachtungsreihe unmöglich wurde, da ungünstige Kulturverhältnisse, denen das Objekt nicht gewachsen war, dessen Tod herbeigeführt haben. Als Kulturgefäß diente ein größeres Uhrglas, welches täglich mit frischem Flußwasser und neuer, aus Infusorien bestehender Nahrung beschickt wurde. Eine einzige Ausnahme hiervon ist in dem beigefügten Protokoll zu finden. Nach jeder Teilung wurde nur ein Tochterindividuum weiter kultiviert. Vom Dezember 1897 an habe ich, um für den Fall eines zufälligen Verlustes gleich Ersatz am Versuchsmaterial zu haben, zeitweilig neben dem Hauptindividuum noch ein Nebenindividuum auf besonderem Uhrglas gezüchtet. Ein ausführliches Protokoll, welches die ganze Beobachtungsreihe besonders mit Rücksicht auf die stattgefundenen Teilungen darstellen soll, ist am Ende dieser Arbeit beigefügt. Es mag daraus hier nur jene Phase herausgegriffen und näher geschildert werden, wo zweikernige Formen zum Vorschein kamen.

Ein einkerniges Individuum, vom 15. November 1897 an täglich beobachtet, wurde am 20. Nov. d. J. in zwei kleinere einkernige und einen größeren zweikernigen Nachkommen geteilt vorgefunden. Dieser zweikernige geriet dann weiter in Kultur, neben ihm aber auch (natürlich im besonderen Uhrglas) einer von den beiden einkernigen Descendenten. Unter den sonst übereinstimmenden Verhältnissen fand ich am 23. November folgendes. An dem zweikernigen Versuchstier hat eine Dreiteilung stattgefunden, die zu zwei kleineren einkernigen und einem größeren zweikernigen Tochterindividuum führte. Es wurde nur letzteres allein in Kultur genommen, bis am 28. November d. J. an ihm abermals eine stattgefundene Dreiteilung sich wahrnehmen ließ, die zwei kleinere, jedoch ungleiche, und ein größeres zweikerniges Tochterindividuum lieferte. Fortgesetzte Kultur dieses letzteren allein ergab dann am 2. Dezember d. J. vier einkernige Nachkommen, von denen nur einer isoliert und weiter kultiviert wurde, um zwei einkernige Teilungsprodukte zu liefern. Dieser Teilungsmodus blieb von jetzt an auch bei den folgenden Generationen bis zum 20. Dezember d. J. erhalten. Die betreffenden Teilungen wurden am 5., 8., 11., 13., 15. und 20. Dezember d. J. konstatiert.

Aus den in diesem Abschnitt angeführten Beobachtungen läßt sich folgender Schluß ziehen: Die mehrkernigen Formen kommen dann zum Vorschein, wenn sich das Medium irgendwie verändert, nämlich wenn, soweit bisher sichergestellt wurde, Mangel oder umgekehrt Überfluß an Nahrung stattfindet, ferner wenn die Kulturen älter werden, und schließlich, wenn eine ungünstige Jahreszeit eintritt.

II.

Eine mehrkernige Form kann entweder durch Kernvermehrung aus einer einkernigen oder durch Verschmelzung der einkernigen Formen miteinander entstehen.

Belege für die erstere Art habe ich im vorhergehenden angeführt, betreffs des zweiten Modus mag ausführlicher folgendes berichtet werden.

Ein einkerniges Individuum, am 19. Mai 1899 einer Glascylinderkultur entnommen, wurde in ein täglich mit frischem Wasser nebst reichlicher Nahrung versehenes Uhrglas überführt. Am Glase haben sich bald Vorticellen angesiedelt, die der Amöbe zur Beute fielen. Am 20. Mai ließ sich die erste Teilung wahrnehmen, deren beide Produkte auf Uhrgläsern weiter kultiviert wurden. Tags darauf fand ich nur eins von den beiden Individuen geteilt, das andre war ungeteilt und an diesem allein setzte ich die Kultur fort. Wie die Untersuchung am 23. Mai ergab, lieferte es abermals nur zwei einkernige Nachkommen, welche weiter gezüchtet wurden. Tags darauf, nach erfolgter Durchmusterung, habe ich sie wieder in das Uhrglas überführt, um die Kultur fortzusetzen. Hierbei traf es sich, daß die beiden Schwesterindividuen einander hart berührten, um gleich darauf miteinander zu verschmelzen. Die am folgenden Tage (nach 24 Stunden) vorgenommene Durchsicht ergab an dem copulierten Objekt die Anwesenheit von zwei Kernen und ebensoviel kontraktilen Vacuolen. Bei fortgesetzter Kultur konnte ich dann am selbigen Objekt (am 27. Mai) eine Dreiteilung sicherstellen, deren zwei Produkte als einkernig, das dritte aber als zweikernig sich erwies. Alle drei Nachkommen wurden dann weiter gezüchtet, der zweikernige anfangs in einem separaten, später jedoch im alten Uhrglase. Die einkernigen Individuen und deren Nachkommen teilten sich stets in zwei einkernige, und was das betreffende zweikernige Individuum betrifft, so fand ich am 2. Juni, daß aus ihm vier Individuen entstanden sind, welche durchwegs nur einen Kern enthielten.

III.

A. Infolge der Mehrkernigkeit wird das Protoplasma komplizierter.

Bildung von neuem Protoplasma ist von der Anwesenheit und Tätigkeit des Kerns abhängig. Fassen wir zunächst den einfachsten Fall ins Auge, nämlich zweikernige Formen, welche wachsen, um mit der Zeit zwei einkernige Nachkommen zu liefern, so darf angenommen werden, daß das neue Protoplasma, welches unter dem Einfluß des einen Kerns entstanden ist, nicht ganz identisch ist mit jenem Protoplasma, welches unter dem Einfluß des andern Kerns entstanden ist. Schon der Umstand, daß die Kerne im Protoplasma ungleiche Lage einnehmen, scheint für einen ungleichen Einfluß ihrerseits auf das neu entstehende Protoplasma zu sprechen. Dazu kann noch der Fall hinzukommen, daß die Kerne deutlich, was ihre Gestalt betrifft, voneinander sich unterscheiden. Hierher gehört der in meiner zitierten Arbeit angeführte Fall, welcher ein Individuum betrifft, dessen einer Kern kleiner, bikonkav, der andre aber größer, tief eingekerbt war. Aus dem ungleichen Bau der Kerne läßt sich dann auf einen ungleichen Wert ihrer Tätigkeit und infolgedessen auch auf einen verschiedenen Einfluß auf das Entstehen von neuem Protoplasma schließen. Beide durch Teilung eines zweikernigen Individuums entstandenen Nachkommen werden demnach zweierlei Protoplasma enthalten, einerseits solches, welches unter dem Einfluß des ersten, anderseits solches, welches unter dem des zweiten Kerns entstanden ist. Infolgedessen wird aber ihr Protoplasma jedenfalls komplizierter sein als bei jenen Individuen, welche durch Teilung eines einkernigen Individuums entstanden sind.

B. Die Mehrkernigkeit führt in der Folge zu einer mehr oder minder großen Verschiedenheit der aus den mehrkernigen durch Teilung entstandenen einkernigen Formen.

Außer dem Einfluß der Kerne auf das Protoplasma muß auch umgekehrt die Wirkung des Protoplasmas auf die Kerne, besonders auf das Wachstum derselben vor ihrer Teilung, erwogen werden. Auch dieser Einfluß braucht keineswegs für einen jeden Kern identisch zu sein. Denn fassen wir wieder eine zweikernige Form, deren Kerne schon ursprünglich ungleich sind und zur nächsten Teilung durch Wachstum sich anschicken, ins Auge, so muß unter dem ungleichen Einfluß des Protoplasmas ihre Differenz noch mehr gesteigert werden, was auch an den durch Teilung aus ihnen entstandenen neuen Kernen zum Ausdruck kommen muß.

Infolge der Mehrkernigkeit wird das Protoplasma komplizierter und ursprünglich gleiche Kerne können sich differenzieren, während ursprünglich ungleiche Kerne noch ungleicher werden können. Somit resultiert aus der Teilung der mehrkernigen Formen eine mehr oder minder große Differenz der so entstandenen neuen einkernigen Individuen. In meiner zitierten Arbeit habe ich dafür zahlreiche Belege angeführt. Die am meisten in die Augen springenden Fälle sind jene, wo z. B. eine zweikernige Form durch Teilung neben einem zweikernigen zwei einkernige, ungleich große Individuen lieferte, oder wo sie durch einen andern Teilungsmodus vier ungleiche Nachkommen erzeugte.

Jene durch Vermittlung der mehrkernigen Formen stattfindende Art der Vermehrung, welche bei den niedrigsten Protozoen vorkommt, möge als Plasmodiogonie bezeichnet werden. Diese ist es allein, der diese Zeilen gelten, und sie mag folgendermaßen definiert werden: Eine Vermehrungsart, bei welcher einkernige Individuen, durch einfache Teilung einkernige Nachkommen liefernd, mit der Zeit zur Entstehung von mehrkernigen Formen den Anlaß geben, welch letztere jedoch mit der Zeit wieder bei ihrer Teilung einkernige Nachkommen ergeben.

Diese Vermehrungsart bietet gewissermaßen das, was der Zweck der geschlechtlichen Vermehrung ist, nämlich eine größere Differenz (Variabilität) der Nachkommen. Sie kommt bei veränderten Verhältnissen des Milieus vor und gibt die Möglichkeit an die Hand, daß den veränderten Verhältnissen durch größere Mannigfaltigkeit der Nachkommen widerstritten wird.

C. Neben den Vorteilen, den der mehrkernige Zustand bietet, kann er auch zum Ausgangspunkt gewisser Nachteile werden.

Haben wir nochmals den einfachsten Fall im Sinn, nämlich den zweikernigen Zustand, so kann der Einfluß des Protoplasmas auf den einen Kern günstiger sein als auf den zweiten. Es kann zum Kampfe zwischen beiden Kernen kommen, in welchem der minder tüchtige Nucleus insofern beeinträchtigt wird, als bei ihm oder bei seinen Nachkommen die Fähigkeit der Vermehrung durch Teilung abhanden kommt. Auf Grund dessen kann man gewisse Fälle erklären, welche mir bei der Untersuchung der mehrkernigen Formen von *Amoeba proteus* vorgekommen sind. Ich meine das Auftreten von einkernigen durch Teilung der mehrkernigen entstandenen Formen, welche deutliche Anzeichen ihres baldigen Unterganges verrieten. Ebenso lassen sich hierdurch jene Fälle aufklären, wo der mehrkernige Mutterorganismus durch Teilung auf der einen Seite teilungsfähige Formen lieferte, auf der andern Seite jedoch daneben auch mehrkernige Nachkommen produzierte, welche zwar einige Zeitlang am Leben blieben, dann aber ohne sich geteilt zu haben zugrunde gingen. Die hier gemeinten Fälle habe ich in meiner zitierten Arbeit geschildert. Nach den dort angeführten Belegen ist es klar, daß bei den mehrkernigen Formen es zu einer solchen Differenz der Kerne kommen kann, daß nur einige Kerne ihre Teilungsfähigkeit unbeschränkt behalten, während bei andern diese Fähigkeit nach einigen Generationen oder überhaupt verloren geht und ihre Tätigkeit in diesem zweiten Falle auf andre vitale Funktionen allein beschränkt bleibt. Wie man sieht, kann bei den mehrkernigen Formen von *Amoeba proteus*, was die Kerne betrifft, derjenige Zustand angedeutet werden, welcher charakteristisch bei höheren Protozoen, nämlich bei Infusorien, ausgeprägt ist. Noch ein Umstand ist hierbei von Wichtigkeit. Wenn mehrkernige Formen zum Teil vermehrungsfähige, zum Teil aber solche Nachkommen liefern, denen diese Fähigkeit in beschränktem Maße oder überhaupt nicht zukommt, so muß man darin Spuren jener Zellendifferenzierung erkennen, welche für mehrzellige Tiere charakteristisch ist, nämlich die Differenzierung in der Vernichtung geweihte und der Vermehrung dienende Zellen.

IV.

A. Sehen wir von mehrkernigen, durch Verschmelzung der einkernigen entstandenen Formen ab, so entstehen die mehrkernigen Formen aus einkernigen in der Weise, daß der ihnen zukommende Kern sich durch Teilung vermehrt, ohne daß zugleich auch das Protoplasma sich teilt. Bei *Amoeba proteus* kann aber noch ein einfacherer Fall vorkommen. Bei den einkernigen Formen kann der Kern nämlich eine Zeitlang durch Wachstum unter Vermehrung seiner Chromatinsubstanz über das sonst übliche Maß sich vergrößern, um erst später gleichzeitig mit dem Plasma sich zu teilen.

Diesen Fall habe ich auf S. 112, Abschnitt B geschildert. Hier hat sich ein einkerniges Individuum nicht in der einfachen Weise geteilt wie seine Schwesterindividuen, sondern sein Kern vergrößerte sich unter Zunahme seines Chromatingehaltes, worauf das Individuum erst später gleichzeitig in zwei einkernige und einen zweikernigen Nachkommen zerfiel, welch letzterer dann mit der Zeit vier einkernige Nachkommen lieferte. Auf Grund dieses angeführten Beispiels kann geschlossen werden, daß der Zustand der vermehrten Kernsubstanz unter ähnlichen Verhältnissen entsteht wie die mehrkernigen Zustände und daß derselbe in ähnlicher Weise wie dort zu einer größeren Komplikation des Protoplasmas und zugleich zur Differenzierung der aus ihm entstandenen Individuen führt.

Um nach gewissen Erscheinungen bei dem eingehenden Studium der Kernstruktur zu schließen, so halte ich dafür, daß bei *Amoeba proteus* auch die Chromatinelemente untereinander nicht gleichwertig sind. Es kann sich ereignen, daß auch bei einfacher Teilung der einkernigen Form die neu entstandenen Kerne nicht ganz gleich zu sein brauchen, indem ihnen ein Chromatingehalt von differentem Werte zukommt. Bei den Formen mit vergrößertem Kern und mit vermehrtem Chromatingehalt kann die Differenz der Chromatinelemente, infolge ihrer veränderten Verteilung im Innern des Kerns und des infolgedessen nicht ganz gleichen Einflusses seitens des Protoplasmas auf sie, noch gesteigert werden. Unter solchen Bedingungen kann es passieren, daß Individuen, welche einem andern Individuum mit vergrößertem Kern ihren Ursprung verdanken, Kerne enthalten, welche mehr oder minder verschiedenen Chromatingehalt aufweisen.

B. Bei *Amoeba proteus* können sich sogar Fälle ereignen, die einen Übergang zwischen Mehrkernigkeit und Kernvergrößerung bilden.

Es möge diesbezüglich folgender Beleg angeführt werden.

Am 8. Juli 1904 wurde aus einer älteren Kultur ein dreikerniges Individuum herausgeholt und für sich gezüchtet. Es wurde täglich frisches Flußwasser nebst neuer Nahrung zugesetzt, wobei eine Durchmusterung der Kultur jeden Tag stattfand. Ich fand am 10. Juli das Resultat einer Vierteilung, wobei zwei Individuen als kleiner und zwei als größer sich erwiesen. Die beiden kleineren Nachkommen besaßen je einen Kern von normaler Größe und Gestalt, während der eine größere Nachkomme zwei ebenfalls normale Kerne enthielt, der andre aber bloß einen zweifachen, d. h. einen Kern, der aus einem größeren und einem kleineren Teile bestand. Beide größeren Individuen wurden weiter gezüchtet. Am 13. Juli d. J. fand ich, daß das Individuum, welches den zweifachen Kern besaß, inzwischen vier gleich große einkernige Nachkommen mit sämtlich normalen Kernen lieferte. Das zweikernige Schwesterprodukt hat sich ebenfalls inzwischen geteilt und zwar in zwei Teile, deren jeder einen zweifachen Kern besitzt, nämlich einen Kern mit einer einseitigen tiefen Striktur. Diese beiden Individuen (*A* und *B*) wurden weiter kultiviert, worauf dann am 15. Juli d. J. an jedem eine vollführte Zweiteilung konstatiert wurde. Beide Individuen, die *A* geliefert hatte, hatten einen zweifachen, d. h. eingeschnürten Nucleus, auch bei dem einen von *B* stammenden Nachkommen fand sich ein ähnlicher Doppelkern vor, aber bei dem zweiten Nachkommen von *B* war ein großer, jedoch vollkommen runder Kern zugegen. Diese beiden letzteren Nachkommen von *B* (*C* und *D*) wurden dann weiter kultiviert. Am folgenden Tage habe ich sichergestellt, daß beide eine Zweiteilung erfahren haben. Immer hatte das eine von beiden Schwesterprodukten einen großen, jedoch runden, das andre einen zweifachen, eingeschnürten Kern. Von diesen vier Nachkommen wurden nur zwei, nämlich die von *D* (*E* und *F*), weiter kultiviert und wie die Durchsicht am 17. Juli d. J. ergab, hat sich das Individuum *F* (mit dem Doppelkern) in zwei einkernige Nachkommen mit normalen Kernen geteilt. Am andern Tage fand ich auch das zweite Individuum (*E*) geteilt und zwar auf zwei Nachkommen, deren einer einen normalen Kern besaß, der andre aber einen dreifachen Kern. Es wurde nur dieses Individuum weiter gezüchtet. Die weiteren Ergebnisse, welche nach einer andern Richtung hin interessant waren, beabsichtige ich an einer andern Stelle zu veröffentlichen.

Man kann den Gedanken eines folgenden Lebenscyclus von einem amöbenartigen Geschöpf entwickeln.

Eine einkernige Form mit vergrößertem Nucleus und mit vermehrtem Chromatingehalt teilt sich so, daß aus ihr drei einkernige Formen (eventuell eine einkernige und eine zweikernige) entstehen, welche dem Untergange geweiht sind, daneben aber eine vierte einkernige Form, deren Vermehrungstätigkeit erhalten bleibt. Diese einkernige Form gibt dann weiter zum Entstehen einer mehrkernigen Form die Veranlassung, indem ihr Kern sich durch Teilung vermehrt, oder daß diese Form mit einer andern einkernigen eine Verschmelzung eingeht. Die mehrkernige Form gibt dann durch Teilung einesteils Formen, welche, ohne sich abermals zu teilen, zugrunde gehen, oder bei denen die Teilungsfähigkeit nach einigen Generationen erlischt, andernteils eine einkernige Form, welche ihren Kern vergrößert, dessen Chromatingehalt vermehrt und so wieder den hier geschilderten Lebenscyclus von neuem beginnt.

Ich halte es für möglich, daß ein solcher Lebenscyclus unter bestimmten Verhältnissen, vielleicht sogar im Versuchswege bei *Amoeba proteus* stabilisiert werden könnte, oder daß derselbe bei einem andern amöbenartigen Organismus unter natürlichen Verhältnissen wirklich vorkommt.

Die Analyse von einem Lebenscyclus solcher Art verspricht überaus anregend auf die Lösung des Rätsels zu wirken, wie mehrzellige Organismen und deren Geschlechtszellen entstanden sind und welche Bedeutung den Erscheinungen zukommt, die bei der Reifung der Geschlechtszellen zutage treten.

Protokoll

betreffend die tägliche Beobachtung der kultivierten *Amoeba proteus* vom 21. Dezember 1896 bis zum 21. Dezember 1897, dann vom 21. Dezember 1897 bis zum 24. August 1898, und enthaltend sämtliche zur Beobachtung gekommene Teilungen.

I.

Datum	Teilung	Datum	Teilung	Datum	Teilung
21. Dez. 1896		21. Januar		21. Februar	
22. -		22. -	IX.	22. -	
23. -		23. -		23. -	XIX.
24. -	I.	24. -		24. -	
25. -		25. -	X.	25. -	XX.
26. -		26. -		26. -	
27. -	II.	27. -		27. -	XXI.
28. -		28. -	XI.	28. -	
29. -		29. -		1. März	XXII.
30. -		30. -		2. -	XXIII.
31. -		31. -		3. -	
1. Jan. 1897	III.	1. Februar	XII.	4. -	
2. -		2. -		5. -	XXIV.
3. -		3. -		6. -	
4. -		4. -	XIII.	7. -	XXV.
5. -	IV.	5. -		8. -	
6. -		6. -		9. -	XXVI.
7. -		7. -	XIV.	10. -	
8. -		8. -		11. -	
9. -		9. -		12. -	XXVII.
10. -	V.	10. -		13. -	
11. -		11. -	XV.	14. -	XXVIII.
12. -		12. -		15. -	
13. -		13. -		16. -	
14. -	VI.	14. -	XVI.	17. -	
15. -		15. -		18. -	XXIX.
16. -		16. -		19. -	
17. -	VII.	17. -	XVII.	20. -	XXX.
18. -		18. -		21. -	
19. -	VIII.	19. -		22. -	
20. -		20. -	XVIII.	23. -	

Datum	Teilung	Datum	Teilung	Datum	Teilung
24. März	XXXI.	11. Mai	LV.	28. Juni	LXXXIX.
25. -	XXXII.	12. -		29. -	XC.
26. -		13. -	LVI.	30. -	XCI.
27. -	XXXIII.	14. -		1. Juli	XCII.
28. -		15. -		2. -	XCIII.
29. -	XXXIV.	16. -	LVII.	3. -	XCIV. u. XCV.
30. -		17. -			
31. -		18. -	LVIII.	4. -	
1. April	XXXV.	19. -		5. -	XCVI.
2. -		20. -	LIX.	6. -	XCVII.
3. -	XXXVI.	21. -	LX.	7. -	
4. -		22. -	LXI.	8. -	XCVIII.
5. -	XXXVII.	23. -	LXII.	9. -	XCIX.
6. -		24. -		10. -	C.
7. -	XXXVIII.	25. -	LXIII.	11. -	CI.
8. -		26. -		12. -	CII.
9. -		27. -	LXIV.	13. -	
10. -	XXXIX.	28. -	LXV.	14. -	CIII.
11. -		29. -	LXVI.	15. -	
12. -		30. -		16. -	CIV.
13. -	XL.	31. -	LXVII.	17. -	CV.
14. -		1. Juni	LXVIII.	18. -	
15. -	XLI.	2. -	LXIX.	19. -	CVI.
16. -		3. -		20. -	CVII.
17. -	XLII.	4. -	LXX.	21. -	CVIII.
18. -		5. -	LXXI.	22. -	CIX.
19. -		6. -	LXXII.	23. -	CX.
20. -	XLIII.	7. -	LXXIII.	24. -	CXI.
21. -	XLIV.	8. -		25. -	
22. -		9. -	LXXIV.	26. -	CXII.
23. -	XLV.	10. -	LXXV.	27. -	CXIII.
24. -	XLVI.	11. -	LXXVI.	28. -	CXIV.
25. -	XLVII.	12. -		29. -	CXV.
26. -		13. -	LXXVII.	30. -	
27. -		14. -	LXXVIII.	31. -	CXVI.
28. -	XLVIII.	15. -	LXXIX.	1. August	
29. -	XLIX.	16. -		2. -	CXVII.
30. -		17. -	LXXX.	3. -	CXVIII.
1. Mai	L.	18. -	LXXXI.	4. -	CXIX.
2. -	LI.	19. -	LXXXII.	5. -	
3. -		20. -	LXXXIII.	6. -	CXX.
4. -	LII.	21. -		7. -	CXXI.
5. -		22. -	LXXXIV.	8. -	CXXII.
6. -	LIII.	23. -	LXXXV.	9. -	CXXIII.
7. -		24. -		10. -	CXXIV.
8. -	LIV.	25. -	LXXXVI.	11. -	CXXV.
9. -		26. -	LXXXVII.	12. -	CXXVI.
10. -		27. -	LXXXVIII.	13. -	CXXVII.

Datum	Teilung	Datum	Teilung	Datum	Teilung
14. August	CXXVIII.	27. Sept.	CLIV.	10. Nov.	
15. -	CXXIX.	28. -	CLV.	11. -	CLXXIV.
16. -		29. -	CLVI.	12. -	
17. -	CXXX.	30. -		13. -	
18. -	CXXXI.	1. Oktober	CLVII.	14. -	
19. -	CXXXII.	2. -		15. -	CLXXV.
20. -	CXXXIII.	3. -	CLVIII.	16. -	
21. -	CXXXIV.	4. -	CLIX.	17. -	
22. -		5. -		18. -	
23. -		6. -	CLX.	19. -	
24. -		7. -		20. -	CLXXVI. u.
25. -	CXXXV.	8. -	CLXI.		CLXXVII.
26. -	CXXXVI.	9. -		21. -	
27. -		10. -		22. -	
28. -	CXXXVII.	11. -	CLXII.	23. -	CLXXVIII.
29. -	CXXXVIII.	12. -		24. -	
30. -		13. -	CLXIII.	25. -	CLXXIX.
31. -	CXXXIX.	14. -		26. -	
1. Sept.		15. -	CLXIV.	27. -	
2. -	CXL.	16. -		28. -	CLXXX.
3. -	CXLI.	17. -	CLXV.	29. -	
4. -		18. -		30. -	
5. -	CXLII.	19. -	CLXVI.	1. Dez.	CLXXXI.
6. -		20. -		2. -	
7. -	CXLIII.	21. -		3. -	
8. -	CXLIV.	22. -		4. -	CLXXXII.
9. -		23. -	CLXVII.	5. -	
10. -	CXLV.	24. -		6. -	
11. -		25. -	CLXVIII.	7. -	CLXXXIII.
12. -	CXLVI.	26. -		8. -	
13. -		27. -		9. -	
14. -	CXLVII.	28. -	CLXIX.	10. -	CLXXXIV.
15. -		29. -		11. -	
16. -	CXLVIII.	30. -		12. -	
17. -		31. -	CLXX.	13. -	CLXXXV.
18. -	CXLIX.	1. Nov.		14. -	
19. -	CL.	2. -		15. -	
20. -		3. -	CLXXI.	16. -	CLXXXVI.
21. -	CLI.	4. -		17. -	
22. -		5. -		18. -	
23. -		6. -		19. -	CLXXXVII.
24. -	CLII.	7. -	CLXXII.	20. -	
25. -	CLIII.	8. -		21. -	CLXXXVIII.
26. -		9. -	CLXXIII.		

II.

Datum	Teilung	Datum	Teilung	Datum	Teilung
21. Dez. 1897		4. Februar		21. März	XXXII.
22. -		5. -	XV.	22. -	
23. -		6. -		23. -	
24. -	I.	7. -		24. -	XXXIII.
25. -		8. -		25. -	
26. -		9. -	XVI.	26. -	XXXIV.
27. -		10. -		27. -	
28. -	II.	11. -		28. -	
29. -		12. -	XVII.	29. -	XXXV.
30. -		13. -		30. -	XXXVI.
31. -	III.	14. -		31. -	
1. Jan. 1898		15. -		1. April	XXXVII.
2. -		16. -		2. -	
3. -	IV.	17. -	XVIII.	3. -	XXXVIII.
4. -		18. -		4. -	
5. -		19. -	XIX.	5. -	
6. -	V.	20. -		6. -	XXXIX.
7. -		21. -		7. -	
8. -		22. -	XX.	8. -	
9. -	VI.	23. -		9. -	
10. -		24. -	XXI.	10. -	XL.
11. -	VII.	25. -		11. -	XLI.
12. -		26. -		12. -	XLII.
13. -		27. -	XXII.	13. -	
14. -	VIII.	28. -		14. -	
15. -		1. März		15. -	XLIII.
16. -		2. -	XXIII.	16. -	
17. -	IX.	3. -		17. -	XLIV.
18. -		4. -	XXIV.	18. -	XLV.
19. -		5. -		19. -	
20. -	X.	6. -	XXV.	20. -	
21. -		7. -		21. -	
22. -		8. -	XXVI.	22. -	XLVI.
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[1] Am 13. August 1898 wurde als Nahrung eine Infusorienkultur benutzt, deren Flüssigkeit schon im Stadium einer ziemlich weitgehenden Zersetzung sich befand. Da es zu befürchten war, daß diese Kultur auf das Versuchstier schädlich einwirken könnte, wurde ein zweites Individuum (Schwesterindividuum des ersteren) in ein andres, bloß mit frischem Flußwasser gefülltes Gefäß überführt. Am 15. d. M. wurde das Individuum im Uhrglas nicht mehr vorgefunden, dafür aber blieb das zweite im Cylindergefäß erhalten und ging inzwischen eine Teilung ein. Von da ab wurde einer von den Nachkommen im Uhrglas wieder unter den üblichen Verhältnissen der Kultur weiter gezüchtet.

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ÜBER DIE

INTRACELLULARE AGGLUTINATION UND VERWANDTE ERSCHEINUNGEN BEI PELOMYXA

4

UND

ANDEREN AMOEBENARTIGEN ORGANISMEN.

III. MITTEILUNG.

VON

ANTONÍN ŠTOLC.

SEPARATABDRUCK AUS DEN SITZUNGSBERICHTEN DER KÖNIGL. BÖHM.
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II.

Über die intracellulare Agglutination und verwandte Erscheinungen bei Pelomyxa und anderen amoebenartigen Organismen.

III. Mitteilung

von **Antonín Štolc.**

Vorgelegt in der Sitzung am 28. Oktober 1910.

Gegenstand dieser Mitteilung bilden die im Protoplasma der *Pelomyxa* und anderer amoebenartigen Organismen sich vorfindenden Sand- oder Quarzkörchen. Ihre Bedeutung habe ich bei der *Pelomyxa* des näheren studiert und erstatte darüber hiemit kurzen Bericht.

Dass diese Sandkörnchen tatsächlich Quarzkörnchen oder in hemischer Beziehung *Siliciumdioxyd* sind, davon überzeugt uns die ach den geläufigen Methoden durchgeführte Prüfung ihrer physikaischen und chemischen Eigenschaften; der Kürze halber will ich es edoch nicht des näheren ausführen.

Diesen Körnchen muss im Leben der *Pelomyxa* und gewiss uch im Leben anderer amoebenartigen Organismen, bei denen sie ich vorfinden, eine wichtige Rolle zukommen. Diese Ansicht schöpfe ch aus meinen langjährigen, bei der *Pelomyxa* gewonnenen Erfahrungen. Bei derselben kommen die Quarzkörnchen im Protoplasma ständig or und bilden somit einen Bestandteil, der im Protoplasma stets eingeschlossen ist. — Eine Periode natürlich ausgenommen, die mit der Fortfplanzungstätigkeit zusammenhängt. Es ist dies die Encystationseriode, oder jene Periode, im welcher die Schutzhülle (Cyste) gebildet wird, und dabei der Körper der *Pelomyxa* in mehrere Teile sich spaltet.

Wie ich in meiner diesbezüglichen Mitteilung*) bereits dargelegt habe, enthält der Körper der zur Bildung von Schutzhülle sich vorbereitenden *Pelomyxa* weder Nahrung noch Nahrungsreste, sondern nur Kerne, Glanzkörper und symbiotische Bakterien. Er enthält daher auch keine Quarzkörnchen, und wir müssen annehmen, dass die Sandkörnchen, die sich früher im Protoplasma befanden, vor der Encystation ausgeschieden wurden, und wohl unter jenen Teilchen zu finden sind, die der äusseren, schleimartigen Partie der Schutzhülle anhaften.

Ein eigentümliches, nach unserer Ansicht mit Rücksicht auf ihre Bedeutung sehr wichtiges Verhalten zeigen die Quarzkörnchen während der Zeit, wo die *Pelomyxa* hungert. Diese Erscheinung habe ich bereits in meiner Abhandlung über die Verdauung und Bildung von Kohlenhydraten bei *Pelomyxa* erwähnt, und zwar an jener Stelle**), wo ich das Schicksal von Individuen der *Pelomyxa* schildere, die in der Natur gesammelt und im Kulturglas solange aufbewahrt wurden, bis in demselben die Nahrung gänzlich erchöpft wurde.

Während dieser Periode des Hungerns befinden sich die Individuen der *Pelomyxa* am Boden des Kulturglases im Schlamme eingesenkt, ihr Körper ist kugelförmig zusammengezogen, von blassweisser Farbe und unbeweglich. Er enthält weder Nahrung noch Nahrungsreste, sondern nur Kerne, erschöpfte Glanzkörper, symbiotische Bakterien und einen Klumpen von zusammengehauften Quarzkörnchen.

Wenn die *Pelomyxa* hungert, findet bekanntlich eine sichtliche Agglutination erschöpfter Glanzkörper und die Agglutination der Kerne statt. Auch die Quarzkörnchen gruppieren sich dabei zugleich zu einer Masse, die bei grösseren Exemplaren der *Pelomyxa* schon mit freiem Auge sichtbar ist, als eine kugelige, vom dem übrigen blassweissen Körper der *Pelomyxa* sich unterscheidende Masse.

In jenen Exemplaren der *Pelomyxa*, die mit Nahrung versorgt, dem Hungern daher nicht ausgesetzt sind, finden sich die Quarzkörnchen wiederum zerstreut im Protoplasma lagernd vor.

---

*) Über die Cystenbildung bei *Pelomyxa*. (Résumé des böhm. Textes) (Sitzungsberichte der königl. böhmischen Gesellschaft der Wissenschaften, 1910.)

**) Beobachtungen u. Versuche über die Verdauung u. Bildung der Kohlenhydrate bei einem amoebenartigen Organismus, Pelomyxa palustris Greeff (Zeitschrift für wissenschaftliche Zoologie, 68 B., 1900, Seite 631).

Es ergibt sich nun die Frage, ob das ebenerwähnte Zusammenhaufen von Quarzkörnchen derselben Reihe von Erscheinungen angehört, wie die Agglutination der Glanzkörper und Kerne, sowie verwandte Erscheinungen bei der *Pelomyxa.* Schon die Beobachtung, dass die Kanten vieler im Protoplasma der *Pelomyxa* befindlichen Quarzkörnchen abgestumpft sind, lässt darrauf schliessen, dass die Körnchen zur Zeit, wo sie sich im Protoplasma befinden, irgend welchen Veränderungen unterworfen sind.

Wir nehmen an, dass im Protoplasma der *Pelomyxa* ein Stoff vorkommt, welcher durch die oberflächlichen Teile der Quarzkörnchen absorbiert wird und mit der Hilfe eines anderen, ebenfalls im Protoplasma der *Pelomyxa* befindlichen Stoffes die Quarzkörnchen, wenn auch in minimalem Masse, auflöst.

Wir nehmen, praeziser gesagt, an, dass sich im Protoplasma der *Pelomyxa* ein Ferment, eine **Silikase** befindet, welche in einem Milieu wirkt, das, wie wir in einer der nächsten Mitteilungen beweisen wollen, von sauerer Reaction ist.

Die Wirkung dieser Silikase besteht in einer Auflösung der oberflächlichen Teile der Quarzkörnchen, indem sich nämlich durch einen Hydratationsprozess aus dem Siliciumdioxyd Kieselsäure bildet, welche im aufgelösten Zustande vom Protoplasma resorbiert und von den Molekülen der lebenden Materie gebunden wird.

Es entsteht nun die weitere Frage, welchen Zweck diese Auflösung der Quarzkörnchen im Protoplasma der *Pelomyxa* und die Bindung des Produktes dieses Auflösungsprozesses an die Moleküle der lebenden Materie hat. Wir müssen annehmen, dass sich die *Pelomyxa* auf diese Weise ein Material vorbereitet, welches eine Vorstufe darstellt, aus welcher bei anderen Sarcodinen entweder die äussere Kieselhülle (das äussere Kieselskelett), beziehungsweise das innere Kieselskelett gebildet wird. Dieses Material bildet also bei der *Pelomyxa,* einem der Repräsentanten der niedrigsten Sarcodinen, die Vorstufe des Kieselskeletts oder der Kieselhülle und dient, an die Moleküle der lebenden Materie gebunden, dem Protoplasma als Schutz, in dem er demselben grössere Festigkeit verleiht.

Das Vorhandensein eines im Protoplasma der *Pelomyxa* auf einen so schwer auflösbaren Stoff, wie es das Siliciumdioxyd ist, wirkenden Ferments wird uns nicht so sonderbar erscheinen, wenn wir die verschiedenen Fermente ins Auge fassen, welche das Protoplasma der *Pelomyxa* enthält, unter denen, wie ich in meiner Abhandlung über die Verdauung und Bildung von Kohlenhydraten bei *Pelomyxa* dargelegt habe, sich auch ein Ferment befindet, welches auf einen organischen, sehr schwer auflösbaren Stoff, nämlich die Cellulose wirkt. Zur Zeit, wo die *Pelomyxa* hungert, bedarf das Protoplasma augenscheinlich eines ausgiebigeren Schutzes. Hiedurch wird eine erhöhte Tätigkeit der Silikase hervorgerufen, die sich darin äussert, dass ein Zusammenhaufen von Quarzkörnchen stattfindet, eine Erscheinung, welche zu derselben Reihe von Erscheinungen gehört, wie die gleichzeitig mit dieser Erscheinung bei der *Pelomyxa* vorkommende sichtliche Agglutination von erschöpften Glanzkörpern und von Kernen.

Zur Zeit der Encystation (der Bildung von Schutzhülle) ist das Vorhandensein der Quarzkörnchen im Protoplasma der *Pelomyxa* überflüssig. Der Schutz des Protoplasmas ist auf eine andere Art gesichert, nämlich durch Bildung einer doppelten, chitin- und schleimartigen Schutzhülle. Diese Hülle ist aus einer organischen, vom Protoplasma ausgeschiedenen Materie entstanden und ist ausserdem vielleicht noch verdichtet durch eine unorganische Materie, durch eine Siliciumverbindung, die entstanden ist vom Material, das durch die vermittelnde Tätigkeit der Silikase an die Moleküle der lebenden Materie gebunden wurde.

Wir wollen die Ergebnisse dieser Mitteilung in folgende Sätze zusammenfassen.

Es ist höchst wahrscheinlich, dass im Protoplasma der *Pelomyxa* und auch bei anderen amoebenartigen Organismen sich neben anderen Fermenten ein besonderes Ferment, Silikase, befindet, welches auf die im Protoplasma lagernden Quarzkörnchen einwirkt und dessen Wirksamkeit von einer, der intracellularen Agglutination analogen Erscheinung begleitet ist.

Dieses Ferment löst, wenn auch in minimalem Masse, die Quarzkörnchen auf; aus Siliciumdioxyd wird im Wege der Hydratation Kieselsäure gebildet, welche vom Protoplasma resorbiert, als ein das Protoplasma schützendes und verdichtendes Material wirkt und auf diese Weise eine Vorstufe der Kieselhülle oder des Kieselskeletts bildet.

Die lebende Materie der Organismen kann also eine Siliciumverbindung auf zweifache Art anpassen oder assimilieren: entweder erhält sie das Siliciummaterial unmittelbar, d. h. im Milieu, in dem sie lebt, bereits aufgelöst, oder sie erhält es durch die vermittelnde

Tätigkeit eines besonderen Fermentes, welches aus fester Siliciumverbindung, aus Siliciumdioxyd, beziehungsweise aus einer anderen Modifikation, die im aufgelösten Zustande befindliche Kieselsäure bildet, welche vom Protoplasma resorbiert, an die Moleküle der lebenden Materie gebunden wird. Die assimilierte Siliciumverbindung bleibt entweder in dauerndem Zusammenhange mit dem Protoplasma, indem sie auf diese Weise eine Vorstufe des Skeletts oder der Hülle bildet, wie es bei der *Pelomyxa* der Fall ist, oder sie spaltet sich vom Protoplasma ab und bildet auf diese Weise die äussere Kieselhülle (das äussere Kieselskelett), beziehungsweise das innere Kieselskelett.

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ÜBER DIE INTRACELLULARE AGGLUTINATION UND VERWANDTE ERSCHEINUNGEN BEI PELOMYXA

UND ANDEREN AMOEBENARTIGEN ORGANISMEN.

IV. MITTEILUNG.

VON

ANTONÍN ŠTOLC.

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Über die intracellulare Agglutination und verwandte Erscheinungen bei Pelomyxa und anderen amoebenartigen Organismen.

IV. Mitteilung.

Von
Antonín Štolc.

Vorgelegt in der Sitzung am 15. Dezember 1911.

Gegenstand dieser Mitteilung ist der Versuch, die chemische Beschaffenheit des Milieus im Protoplasma der Pelomyxa zu bestimmen.

Ich hielt es für zweckmässig, die chemische Reaktion im Protoplasma der *Pelomyxa* zu untersuchen, um die Natur des intracellularen Agglutinins und Lysins, sowie die Art ihrer Wirkungsweise im Protoplasma der *Pelomyxa* einigermassen beleuchten zu können.

Ich nahm eine Anzahl von Stoffen bestimmter chemischer Zusammensetzung, brachte dieselben in den Körper der *Pelomyxa* und verfolgte hierauf genau ihr Verhalten im Protoplasma der *Pelomyxa*. Auf diese Weise wurde eine Reihe von Versuchen vorgenommen, die ich hier des näheren nicht erörtern will; das Ergebnis dieser Versuche bildete, wie ich zugleich bemerke, die Vorbereitung zum Hauptversuche, der die Entscheidung bringen sollte.

Zu diesem Versuche benützte ich als Versuchsmaterial einen bestimmten Farbstoff, nämlich die Base des Neutralrot. Diese Base bildet gelbbraune Nadeln, welche bei normaler Temperatur im neutralen oder alkalischen Milieu keine Veränderung aufweisen, wohl aber im geeigneten saueren Milieu. Im geeigneten saueren Milieu lösen sich nämlich diese Nadeln auf, die Base verbindet sich mit einem Stoffe saueren Charakters, welcher das Wesen des Milieus bildet. Auf

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diese Weise ensteht eine Salzverbindung, das Salz dieser Base, welches als roter Farbstoff erscheint.

Der Hauptversuch wurde auf folgende Weise vorgenommen:

Ich nahm eine Anzahl von Exemplaren der *Pelomyxa*, die in einem grossen Versuchsglase gezüchtet waren, wie ich dies in meiner Abhandlung „Ueber die Verdauung und Bildung von Kohlenhydraten bei *Pelomyxa* etc.“*) beschrieben habe. Eine Anzahl dieser Exemplare wurde in einem niedrigen Glasgefäss isoliert, andere dagegen in einem schmalen Glasgefäss, also auf eine Art, wie ich es in der eben angeführten Abhandlung (S. 635) erwähne. Diese Gefässe waren schon früher mit Wasser aus dem grossen Versuchsglase gefüllt worden und zu den darin befindlichen Exemplaren der *Pelomyxa* wurden Nadeln der Base des Neutralrot hinzugefügt. Hierauf wurden beide Gefässe in das große Versuchsglas getaucht, wo sie eine Stunde verblieben. Den Exemplaren der *Pelomyxa* wurde so die Möglichkeit gegeben, sich der Nadeln des Farbstoffes zu bemächtigen und dieselben in ihrem Protoplasma einzuschliessen. Nach Ablauf dieser Zeit wurden die Gefässe wieder aus dem großen Versuchsglas herausgenommen und die in demselben isolierten Exemplare der *Pelomyxa* wurden dann mikroskopisch untersucht.

Ich konstatierte, dass dieselben im Laufe der eben angeführten Zeit einen Teil des ihnen gebotenen Farbstoffes aufgenommen haben. In ihrem Körper fand ich zahlreiche, im Protoplasma dicht eingeschlossene Nadeln des Neutralrot. Beide Gefässe wurden sorgfältigst gereinigt und mit Flusswasser gewaschen; hierauf wurden sie mit Wasser aus dem grossen Versuchsglase gefüllt und sodann wurden in denselben wieder Exemplare der *Pelomyxa* isoliert, die aufgenommene Nadeln des Farbstoffes enthielten. Schliesslich wurden die beiden Gefässe in das grosse Versuchsglas getaucht und darin 24 Stunden belassen. Nach dieser Zeit wurden die isolierten Exemplare der *Pelomyxa* abermals untersucht. Schon mit freiem Auge konnte ich sehen, dass dieselben gefärbt sind. Ihr Körper erscheint in hellem Ziegelrot. Eine genaue mikroskopische Untersuchung ergibt dann, dass das Protoplasma diffus rot gefärbt zu sein scheint, was auf die rote Färbung der feinen, im Protoplasma enthaltenen Körnchen zurückzuführen ist. Gefärbt sind ausserdem auch verschiedene, im Protoplasma verstreut eingeschlossene und dem Verdauungsprozess unterworfene Nahrungspartikelchen. Im Protoplasma finden wir weiters noch Nadeln des aufgenommenen Farbstoffes, jedoch in viel geringerer Zahl als sie zuerst gefunden wurden. An den Nadeln sind Auflösungserscheinungen bemerkbar, und an einigen derselben sind Vacuolen, erfüllt mit einer klaren, durchsichtigen Flüssigkeit, ganz deutlich zu erkennen. Die Vacuolen sind kugelförmig oder in die Länge gezogen, (an beiden Enden verjüngt) und umschliessen entweder ein Ende der Nadel oder deren mittleren Teil.

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*) Beobachtungen und Versuche über die Verdauung und Bildung der Kohlenhydrate bei einem amoebenartigen Organismus, Pelomyxa palustris Greeff. (Zeitschrift für wissenschaftliche Zoologie, Bd. LXVIII, 1900, S. 631 u. f.)

Die im oben geschilderten Zustande vorgefundenen Exemplare der *Pelomyxa* wurden abermals in beiden Gefässen isoliert, und diese Gefässe verblieben in dem grossen Versuchsglase 3mal 24 Stunden. Nach Ablauf dieser Zeit ergab eine neuerliche Untersuchung von Exemplaren der *Pelomyxa* bereits den vollständigen Verlust ihrer Färbung. Bei genauer mikroskopischer Untersuchung fand ich in ihrem Körper nicht eine Spur mehr von Nadeln des Farbstoffes, und das Aussehen des Protoplasma ist wieder dasselbe wie vor der Aufnahme der Nadeln des Farbstoffes.

Kurze Zeit darauf wiederholte ich den Hauptversuch in einer bloss in Nebenumständen etwas abgeänderten Form. Das Resultat stimmte aber vollkommen mit demjenigen des früheren Versuches überein.

Aus dem hier angeführten Hauptversuche geht hervor, dass das Protoplasma der Pelomyxa ein saueres Agens enthält. Dieses Agens befindet sich im Milieu, welches die Moleküle des lebenden Protoplasmas umschliesst, und es verleiht dem Milieu einen bestimmten chemischen Charakter, nämlich den Charakter der saueren Reaktion. Die von der *Pelomyxa* aufgenommenen Nadeln der Base des Neutralrot werden im Protoplasma durch dieses Agens aufgelöst. Dieses Agens vereinigt sich mit der Base, es entsteht eine Verbindung, die in den Vacuolen enthalten ist, welche die der Auflösung unterworfenen Nadeln des Farbstoffes umschliessen, und die in das die Molecüle des lebenden Protoplasmas umschliessende Milieu gelangt und hier das Material darstellt, durch welches die im Protoplasma der *Pelomyxa* enthaltenen Körnchen sowie die im Körper der *Pelomyxa* befindlichen, dem Verdauungsprozesse unterworfenen Nahrungspartikelchen gefärbt werden.

Im Laufe der Zeit findet, wie aus dem Versuche ersichtlich ist, die Entfärbung der Körnchen und Nahrungspartikelchen dadurch statt, dass der Farbstoff aus dem Körper der *Pelomyxa* ausgeschieden wird.

Der Körper der Pelomyxa enthält verschiedene Fermente, die ihre Tätigkeit nur unter Mitwirkung eines Milieus von bestimmter chemischer Reaktion entfalten können. Wir müssen also annehmen, dass die Fermente, von denen wir voraussetzen, das sie in eigenem Protoplasma wirken, wie es zum Beispiel bei der Silikase der Fall ist, die wir in unserer früheren Abhandlung erwähnten, im Milieu wirken, dessen Charakter durch den früher erwähnten Hauptversuch als sauer bestimmt wird. In diesem Milieu sollen auch intracellulare Agglutinine und Lysine ihre Wirkung äussern, und es ist höchst wahrscheinlich, dass auch sie sich in ihrer Zusammensetzung als kompliziert erweisen werden.

VII.

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Über die Teilung des Protoplasmas im mehrkernigen Zustande.

Nach den Untersuchungen an mehrkernigen Formen der Amoeba proteus.

6

Von

Antonín Štolc.

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Eingegangen am 8. Mai 1905.

Diese Arbeit stellte sich zur Aufgabe, auf Grund der Beobachtungen und Experimente zu untersuchen, wie sich die Verhältnisse der Zellkerne und des Protoplasmas gestalten, wenn letzteres sich teilt.

Ein hierzu geeignetes Material fand ich in den mehrkernigen Formen der *Amoeba proteus*. Ich kultivierte die betreffenden Amöben in kleinen Glasgefäßen von zylindrischer Gestalt, welche mit Flußwasser angefüllt waren. Diesem wurden als Nahrung für die Amöben die in besonderen Kulturen gezüchteten Infusorien hinzugefügt. Jeden Tag oder in längeren Zeitpausen, nachdem ein Teil des Kulturwassers weggenommen und mit frischem Wasser ersetzt wurde, fügte ich neue Nahrung hinzu.

Unter solchen Umständen vermehrten sich die Amöben in der Regel eifrig, während in den älteren Kulturen zeitweilig ziemlich häufig mehrkernige Formen auftraten. Gewöhnlich waren letztere schon auf den ersten Blick als solche zu erkennen, da sie in der Regel größer sind als die einkernigen Formen. Am häufigsten kamen zweikernige, minder häufig drei- oder vierkernige Individuen zum Vorschein. Mit den ersteren konnten deshalb die meisten Untersuchungen ausgeführt werden.

In der Mehrzahl von Fällen teilten sich die mehrkernigen Formen, nachdem sie im Flußwasser nach Hinzugabe der Nahrung isoliert und weiter kultiviert wurden. Zu diesem Zwecke wurde dann gewöhnlich Wasser nebst Nahrung täglich erneuert.

Darauf gründete ich meine Untersuchungen, indem ich zugleich bestrebt war, einerseits in verschiedenen Jahreszeiten zu operieren, anderseits eine möglichst lange Beobachtungsreihe, vom Jahre 1897 bis Ende 1904, zu gewinnen.

Was die Kultur der mehrkernigen isolierten Formen betrifft, so fand dieselbe in Uhrgläsern, ausgehöhlten Objektgläsern (Hohlgläschen), oder in Zylindergläschen statt. Die betreffenden Objekte wurden jeden Tag unter dem Mikroskop durchgesehen, wobei Ausnahmen von dieser Regel an den bezüglichen Stellen des Textes angemerkt sind.

Nach dem, was über die Vermehrung der Protozoen im Zustande der Vielkernigkeit bislang bekannt ist, waren bei den mehrkernigen Formen der *Amoeba proteus* folgende Teilungsmodifikationen zu erwarten:

1) Protoplasmateilung ohne gleichzeitige Kernteilung.
2) Protoplasmateilung mit gleichzeitiger Teilung aller Kerne.
3) Protoplasmateilung mit gleichzeitiger Teilung eines Bruchteils der gesamten Kerne.

In bezug auf die zu erwartenden Fälle mögen hier die betreffenden Untersuchungsresultate angeführt und in bestimmte Gruppen geordnet werden.

I.

A. Ein zweikerniges Individuum in einem ausgehöhlten Objektträger seit 31. Oktober 1898 kultiviert, erscheint am 3. November d. J. in zwei einkernige Individuen geteilt.

B. Ein zweikerniges Individuum seit 25. April 1900 im Hohlgläschen, seit 30. April d. J. im Uhrglas kultiviert, erscheint am 1. Mai d. J. in zwei einkernige Tochterindividuen geteilt.

C. Ein zweikerniges Individuum wurde vom 21. Juni 1901 an im Uhrglas gezüchtet. Es besaß ungleiche Kerne, von denen der eine kleiner, linsenförmig, bikonkav, der andre größer, tief eingekerbt war. Am 23. Juni d. J. fand ich zwei einkernige Nachkommen vor, deren Kerne noch deutlich die ursprüngliche Form und Größe (wie im Mutterindividuum) besaßen.

Schlußfolgerung. Aus den eben angeführten Fällen ergibt sich eine von der Kernteilung nicht begleitete Plasmateilung, so daß der Vorgang also dem in der Einleitung sub 1 bezeichneten Typus entspricht.

II.

A. Ein zweikerniges Individuum, seit 24. April 1898 im Uhrglas gezüchtet, erschien am 27. April in drei Individuen geteilt, von denen zwei kleiner, einkernig, das dritte größer, zweikernig war. Fortgesetzte separate Kultur des letzteren ergab am 25. Mai d. J., daß die Amöbe eine kurze Zeit vorher zugrunde gegangen sein mußte, da im Kulturwasser von dem betreffenden lebenden Objekte nichts mehr ausfindig gemacht werden konnte.

B. Ein zweikerniges Individuum, seit 10. Juni 1898 im Uhrglas gezüchtet, erschien am 22. Juni d. J. in vier einkernige Tochterindividuen von ungleicher Größe geteilt.

C. Ein zweikerniges Individuum, vom 5. Oktober 1898 an im Uhrglas kultiviert, ergab am 7. Oktober d. J. drei Nachkommen, zwei kleinere einkernige und einen größeren zweikernigen.

Letzterer, für sich allein in Kultur genommen, erschien am 9. Oktober in vier einkernige Tochterindividuen geteilt.

D. Ein zweikerniges Individuum, welches seit 12. April 1900 im Uhrglas kultiviert wurde, erfuhr eine Vierteilung, indem am 16. April d. J. an seiner Stelle vier einkernige Nachkommen sich vorfanden. Von diesen waren zwei viel kleiner als die beiden andern.

E. Längere Beobachtungsreihe. Ein zweikerniges Individuum, seit 20. April 1900 im Uhrglas kultiviert, erschien am 21. April d. J. in drei Tochterindividuen geteilt, von denen zwei einkernig, das dritte zweikernig war.

Letzteres, separiert und weiter kultiviert, ergab am 25. April drei Nachkommen, zwei einkernige und einen zweikernigen.

Dieser, für sich allein gezüchtet, erfuhr ebenfalls eine Dreiteilung, indem am 30. April abermals zwei einkernige und ein zweikerniges Tochterindividuum im Uhrglas zum Vorschein kamen.

Nachdem noch das letztgenannte zweikernige Individuum unter denselben Bedingungen für sich weiter kultiviert wurde, erschienen schließlich (am 5. Mai) vier, sämtlich einkernige Nachkommen.

F. Ein zweikerniges Individuum, seit 21. April 1900 im Uhrglas gezüchtet, ergab am 23. April d. J. vier neue Individuen mit je einem Kern.

G. Ein zweikerniges Individuum, welches seit 22. April 1900 im Hohlgläschen, vom 23. bis zum 24. April d. J. jedoch im Uhrglas kultiviert wurde, erfuhr ebenfalls, wie die am 24. April stattgefundene

Untersuchung ergab, eine Vierteilung, deren Produkte alle einkernig waren.

H. Ein zweikerniges Individuum, seit 24. April 1900 im Uhrglas gezüchtet, ließ am 25. April statt seiner vier Nachkommen wahrnehmen, welche sämtlich je einen Kern führten.

I. Ein zweikerniges Individuum, welches seit 25. April 1900 in einer Uhrglaskultur sich befand, wurde am 27. April d. J. in einer von den bisherigen abweichenden Weise geteilt vorgefunden. Man sah nämlich zwei Individuen, von denen das eine klein und einkernig, das andre groß und dreikernig war.

K. Ein zweikerniges Individuum, vom 7. Mai 1900 an im Uhrglas gezüchtet, erschien am 9. Mai d. J. in drei Tochterindividuen geteilt, von denen zwei kleinere als einkernig, das dritte, größere, als zweikernig sich erwiesen.

L. Ein zweikerniges Individuum, seit 29. September 1903 im Zylindergläschen kultiviert, erschien am 1. Oktober d. J. in drei Tochterindividuen geteilt, von welchen zwei einkernig, das dritte zweikernig war.

Letzteres allein wurde abermals in Kultur genommen, wobei am 2. Oktober d. J. Nahrung (nicht jedoch frisches Wasser) ausblieb. Da am 4. Oktober d. J. das Objekt im Wasser nicht mehr aufzufinden war, so muß ich annehmen, daß es zugrunde gegangen ist.

M. Ein zweikerniges Individuum nahm ich seit 9. Oktober 1903 in Kultur. In dem betreffenden Kulturgläschen (Zylindergläschen) hat es zwar anfangs wenig Nahrung aufgenommen, doch erschien es am 19. Oktober d. J. mit dieser angefüllt.

Nachdem am 12. Oktober d. J. sowohl die sonst übliche Untersuchung, als auch Hinzugabe von frischem Wasser nebst Nahrung ausgeblieben ist, fand ich am 20. Oktober d. J. das Objekt in vier einkernige Tochterindividuen getrennt.

N. Ein zweikerniges Individuum wurde seit 19. September 1903 im Zylindergläschen gezüchtet. Am 19. und 16. September d. J. unterblieb zwar die Neuhinzugabe von Nahrung, aber nicht die Erneuerung des Kulturwassers. Am 23. September d. J. konnte ich eine Dreiteilung direkt beobachten, der zwei ungleich große, einkernige, und ein zweikerniges Tochterindividuum entstammten. Die neu entstandenen, gleich nach der Teilung untersuchten Kerne erschienen überaus fein gekörnt.

Der zweikernige Descendent, nachdem er sofort in Kultur genommen wurde, erschien am 29. September d. J. in vier einkernige Tochterindividuen geteilt, von denen drei annähernd gleich groß, das vierte kleiner war als die andern.

Bemerkung. Ich habe des öftern Vermehrung von *Amoeba proteus* durch Teilung direkt verfolgt. Die hierbei zutage tretenden Erscheinungen beabsichtige ich bei einer andern Gelegenheit ausführlicher zu schildern. Hier mag nur soviel angeführt werden, daß der Kern vor seiner Teilung grob gekörnt erscheint, indem die Chromatinelemente dieses Aussehen bedingen. Bei Beginn der Teilung wird der Kern fast hyalin flüssig, die neu entstandenen Tochterkerne erscheinen dann anfangs äußerst feinkörnig.

Schlußfolgerung. Aus den angeführten Fällen (A bis M), besonders aber aus der Beobachtung N, ergibt sich, daß überall eine Protoplasmateilung gleichzeitig mit der Teilung sämtlicher Kerne stattgefunden haben mußte, daß also mehrkernige Formen von *Amoeba proteus* auch nach dem sub 2 in der Einleitung charakterisierten Typus sich zu teilen vermögen.

III.

A. Ein zweikerniges Individuum, seit 2. Mai 1900 im Uhrglas gezüchtet, erschien am 4. Mai d. J. in ein einkerniges und ein zweikerniges Tochterindividuum geteilt. Letzteres wurde zwar sofort allein für sich kultiviert, doch traten später an ihm Symptome einer Schwächung zutage, so daß von weiterer Kultur Abstand genommen wurde.

B. Ein zweikerniges Individuum, vom 17. Oktober 1903 an im Zylindergläschen gezüchtet, erfuhr eine Dreiteilung, indem am 7. November d. J. an seiner Stelle drei einkernige Nachkommen sichergestellt wurden.

C. Ein vierkerniges Individuum, seit 28. September 1900 im Uhrglas gezüchtet, ergab am 1. Oktober d. J. drei kleinere einkernige und ein größeres, dreikerniges Teilungsprodukt.

Schlußfolgerung. Aus den in diesem Absatze kurz geschilderten Fällen geht hervor, daß überall Protoplasmateilung eintrat, ohne daß gleichzeitig alle Kerne sich geteilt hätten. Somit kommt den vielkernigen Formen von *Amoeba proteus* ebenfalls auch die Fähigkeit zu, sich nach dem in der Einleitung sub 3 charakterisierten Typus zu teilen.

IV.

A. Ein dreikerniges Individuum wurde seit 3. März 1897 im Uhrglas gezüchtet. Zwei von den Kernen sind von normaler Form, der dritte etwas abweichend gestaltet. Am 11. März d. J. wurde eine bereits erfolgte Dreiteilung sichergestellt, die zu einem dreikernigen, einem zweikernigen und einem einkernigen Tochterindividuum geführt hat. Letzteres führte einen abweichenden, etwas eingeschnürten Kern. Nachdem ich sofort wieder das dreikernige Individuum in Kultur genommen hatte, fand ich dasselbe am 13. März d. J. in fünf Individuen geteilt, von denen vier je einen Kern (darunter ein Individuum kleiner als die übrigen) und das fünfte zwei Kerne besaßen. Letzteres wurde abermals weiter gezüchtet, bis am 16. März d. J. zwei einkernige Nachkommen konstatiert wurden.

Einer von beiden Nachkommen wurde weiter gezüchtet und als er sich in zwei einkernige Descendenten geteilt hat, von diesen einer wieder in Kultur genommen usw. Auf diese Weise wurde die Zweiteilung bis zum 23. März d. J. wiederholt beobachtet.

B. Ein zweikerniges Individuum, seit 24. April 1898 im Uhrglas gezüchtet, erschien am 27. April d. J. in drei Tochterindividuen geteilt, von denen zwei einkernig, das dritte zweikernig war.

Das zweikernige Produkt kam sofort wieder in Kultur, bis am 30. April d. J. die Entstehung von zwei einkernigen Descendenten sichergestellt wurde.

Fortgesetzte Kultur von einem derselben ergab am 1. Mai wieder zwei einkernige Nachkommen.

C. Ein zweikerniges Individuum[1]), seit 5. Oktober 1898 im Hohlgläschen kultiviert, ergab am 12. Oktober d. J. einen einkernigen und einen zweikernigen Nachkommen.

Aus diesem[2]) entstanden am 13. Oktober d. J. wieder ein einkerniger und ein zweikerniger Nachkomme. Letzterer erschien am 22. Oktober d. J. in drei Individuen geteilt, von welchen zwei einkernig und das dritte zweikernig war. Am 26. Oktober d. J. wurde bezüglich des letzteren eine Zweiteilung konstatiert, deren Produkte als zweikernig sich erwiesen. Ein von beiden Produkten hat dann am 30. Oktober d. J. vier einkernige Descendenten geliefert.

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[1]) Das andre durch eine Zweiteilung entstandene Schwesterprodukt von ihm lieferte das Material zu den sub II. C angeführten Beobachtungen.

[2]) Natürlich nach erfolgter Isolierung und bei fortgesetzter Kultur, was der Kürze halber auch im folgenden als selbstverständlich ausgelassen wird.

Bemerkung. Der Fall vom 26. Oktober unterscheidet sich von den schon angeführten Fällen durch die gleiche Verteilung der Zahl der Tochterkerne an die beiden Protoplasmahälften.

D. Eine umfangreichere Beobachtungsreihe. Ein zweikerniges Individuum, seit 3. September 1903 im Zylindergläschen kultiviert, erschien am 4. September d. J. in zwei einkernige, ungleiche, und einen zweikernigen Nachkommen geteilt.

Letzteren fand ich am 8. September d. J. in zwei einkernige, ungleiche, und ein dreikerniges Tochterindividuum geteilt. Dieser von den vorhergehenden abweichende Fall läßt sich so erklären, daß der eine Kern sich im voraus teilte, worauf dann das Individuum im dreikernigen Zustande der Teilung anheimfiel.

Aus dem dreikernigen Individuum entstanden nach der Beobachtung am 9. September d. J. drei Nachkommen, von welchen der eine einkernig, der andre zweikernig und der dritte (größte) dreikernig war. Die beiden letzteren wurden dann weiter gezüchtet.

Am 10. September d. J. erschien das dreikernige Individuum in einen einkernigen und einen dreikernigen Nachkommen geteilt.

Das zweikernige Individuum vom 9. September d. J. hat sich erst spät geteilt und ergab am 14. September d. J. zwei einkernige, ungleiche, und einen zweikernigen Nachkommen.

Bei der Durchsicht des letzteren am 20. September d. J. wurde ein Teil des Protoplasmas durch die Pipette weggerissen. Nachdem dann am 26. September nur das Wasser erneuert wurde, fand ich am 28. September dessen drei Nachkommen von fast gleicher Größe, von denen zwei als einkernig, der dritte als zweikernig sich erwiesen.

Letzterer erscheint am 1. Oktober d. J. in vier einkernige Tochterindividuen geteilt.

Das dreikernige Individuum vom 10. September fand ich am 2. Oktober d. J. in zwei einkernige und zwei zweikernige Nachkommen geteilt. Alle vier wurden weiter gezüchtet.

Am 4. Oktober d. J. habe ich nur das Wasser erneuert. Die zwei einkernigen Nachkommen wurden geteilt vorgefunden.

Am 7. Oktober d. J. ließ sich konstatieren, daß ein von den beiden zweikernigen Individuen vierkernig wurde.

Am 8. Oktober d. J. fand ich das andre zweikernige Individuum in drei einkernige Nachkommen geteilt vor. Von diesen wurde nur ein etwas abweichend gestalteter weiter gezüchtet.

Am 12. Oktober d. J. wurde weder beobachtet noch das Kulturmedium erneuert.

Am 31. Oktober, am 1. und 2. November d. J. fand nur Erneuerung des Wassers statt.

Am 2. November d. J. erschien das vierkernige Individuum (vom 7. Oktober d. J.) in einen (großen) sechskernigen und einen (kleinen) einkernigen Nachkommen geteilt. Der erstere allein wurde dann weiter kultiviert.

Vom 15. November bis 2. Dezember d. J. wurde nur das Kulturwasser erneuert.

Am 22. November d. J. wurde das abweichend gestaltete Individuum (vom 8. Oktober d. J.) nicht mehr angetroffen, woraus geschlossen werden muß, daß es zugrunde gegangen ist.

Am 5. Dezember d. J. konnte das sechskernige Individuum (vom 2. November d. J.) nicht mehr im Kulturwasser entdeckt werden, es mußte also auch dem Tode anheimgefallen sein.

Schlußfolgerung. Aus den im IV. Abschnitt angeführten Beobachtungen ist zu ersehen, daß sich das Protoplasma im mehrkernigen Zustande teils nach dem ersten, teils nach dem zweiten oder dritten Typus geteilt hat.

Für die verschiedenen Modi der Protoplasmateilung im mehrkernigen Zustande ist das Verhalten der Kerne charakteristisch. In bezug auf die Teilungsprodukte, die hierbei entstehen, muß eingestanden werden, daß in der Reihe der angeführten Fälle eine große Mannigfaltigkeit waltet.

Wenn nur das Protoplasma im zweikernigen Zustande in Betracht genommen wird, so finden wir folgende Modifikationen des Teilungsresultates: Das Protoplasma teilt sich in zwei gleiche oder ungleiche einkernige Teile; das Protoplasma wird in zwei zweikernige oder zwei einkernige gleiche oder ungleiche und einen zweikernigen Teil zerklüftet; das Protoplasma zerfällt in einen einkernigen und einen dreikernigen Teil, oder es erfährt eine Vierteilung, deren Produkte gleich oder ungleich sein können und je einen Kern besitzen; das Protoplasma wird in einen zweikernigen und einen einkernigen Teil geschieden, bzw. es entstehen aus ihm drei einkernige Teilungsprodukte.

Gewiß sind noch andre Teilungsresultate des zweikernigen Protoplasmas möglich, als in den von mir beschriebenen Fällen zutage getreten sind.

Die verschiedenen Teilungsprodukte des zweikernigen Protoplasmas lassen sich durch bestimmte Symbole ausdrücken, welche im folgenden der Kürze halber ausschließlich zur Anwendung gelangen.

Der mehrkernige Zustand möge durch eine römische Ziffer (z. B. II, der zweikernige Zustand) bezeichnet werden; eine arabische Ziffer vor der römischen möge die entsprechende Teilungsart (1., 2., 3. Art), die Summe der hinter der römischen Ziffer angebrachten arabischen Ziffern die Zahl der entstandenen Teilungsprodukte, und jede einzelne der letztgenannten arabischen Ziffern die Anzahl der im Teilungsprodukt enthaltenen Kerne andeuten. Schließlich soll das hin und wieder beigefügte u die Ungleichheit der Teile (bei gleicher Kernzahl) zum Ausdruck bringen.

Somit entstehen auf Grund der beobachteten Teilungsresultate des zweikernigen Protoplasmas (der zweikernigen Formen von *Amoeba proteus*) die folgenden Symbole: 1 II 1.1 — 1 II 1.1 u — 2 II 2.2 — 2 II 2.1.1 — 2 II 2.1.1 u — 2 II 3.1 — 2 II 1.1.1.1 — 2 II 1.1.1.1 u — 3 II 2.1 — 3 II 1.1.1.

So bedeutet z. B. Symbol 1 II 1.1: Das im zweikernigen Zustande befindliche Protoplasma hat sich nach dem Typus 1 in zwei einkernige Teile geteilt; Symbol 2 II 2.1.1 u bedeutet, daß das Protoplasma nach dem Typus 2 in einen zweikernigen und zwei einkernige ungleiche Teile zerfiel; Symbol 3 II 2.1 bedeutet den dritten Teilungstypus, wobei eine zweikernige und eine einkernige Portion entstand usw.

Man kann auf das Protoplasma, eventuell auch auf den Kern, durch gewisse Mittel einwirken, welche zwar keinen günstigen Einfluß auf die vitale Tätigkeit ausüben, jedoch letztere keineswegs vernichten. Kommen diese Mittel bei dem mehrkernigen Protoplasma zur Anwendung, so liegt die Frage nahe, ob hierdurch die Teilungsmodi in irgendwelcher Weise betroffen werden, so daß z. B. eine oder die andre Teilungsart stabilisiert oder andre, unter den angeführten Fällen nicht enthaltene Prozesse plötzlich eingeleitet (induziert) würden.

In den folgenden Abschnitten mögen die durch besondere Mittel erzielten Versuchsresultate angeführt werden.

1. Versuch bei beschränkter Nahrung.

Dieser Versuch war leicht auszuführen, indem der mehrkernige Zustand von *Amoeba proteus* nur in Flußwasser, das erfahrungsgemäß sehr nahrungsarm war, gezüchtet wurde. Es ließ sich voraussetzen, daß in diesem Falle keine andern Teilungsmodi auftreten werden, als in den oben beschriebenen Fällen, sondern daß nur das Spatium zwischen zwei consecutiven Teilungen länger sein wird.

Es möge hier kurz der Versuch und sein Resultat angeführt werden.

Ein zweikerniges Individuum wurde im Uhrglas im Flußwasser, welches jeden Tag Erneuerung erfuhr, gezüchtet. Anfang des Versuchs am 5. November 1898. Am 11. November d. J. wurde das Teilungsresultat nach dem Symbol: 3 II 2.1 festgestellt.

Das zweikernige Individuum, auf dieselbe Art gezüchtet wie das Mutterindividuum, ergab am 30. November d. J. folgendes Teilungsresultat, symbolisch ausgedrückt: 1 II 1.1.

Schlußfolgerung. Dem Resultate nach läßt sich schließen, daß die oben angeführte Voraussetzung richtig gewesen.

2. Versuche mit Abtrennung eines Protoplasmateils.

Wird den mehrkernigen Formen von *Amoeba proteus* ein Teil des Protoplasmas (mittels einer scharfen, feinen Nadel) weggenommen, so läßt sich voraussetzen, daß bei sonst gleichen Kulturverhältnissen sowohl die intakten, als auch die eines Plasmateils beraubten Formen sich im großen ganzen gleich verhalten werden, nur mit dem Unterschied, daß die letzteren vielleicht zeitliche Verschiedenheiten in der Aufeinanderfolge der Teilungen aufweisen werden.

Wir fügen eine kurze Übersicht der betreffenden Versuche und deren Resultate bei.

A. Ein zweikerniges Individuum, in der angeführten Weise eines Protoplasmateils beraubt, wurde seit 24. Februar 1898 im Uhrglas gezüchtet. Am 26. Februar d. J. stellte ich das Resultat einer Dreiteilung nach dem Symbol: 2 II 2.1.1 fest. Alle drei Individuen sind von gleicher Größe; nur das zweikernige wird dann weiter gezüchtet.

Am 2. März d. J. ließ sich wieder eine stattgefundene Teilung nachweisen, die der Formel: 2 II 2.1.1 folgte. Nur das zweikernige Teilungsprodukt wurde weiter kultiviert. Am 6. März d. J. abermals eine Teilung von der Formel: 2 II 1.1.1.1.

Einen von den vier Nachkommen habe ich dann weiter gezüchtet, bis eine Zweiteilung stattfand. Von da ab wurden bis zum 11. Mai Zweiteilungen beobachtet, wobei immer nur ein Descendent in Kultur genommen wurde. Anfangs fand eine gleichzeitige Erneuerung von Flußwasser und Nahrung statt, später nur von Wasser. Die Beobachtung ergab den Versuchsbedingungen entsprechend anfangs ein rascheres, später ein langsameres Vermehrungstempo der Amöben.

B. Ein zweikerniges Individuum, dem ein Teil des Protoplasmas weggenommen wurde, kam in ein Hohlgläschen zur Kultur. Der Versuch begann am 1. April 1898.

Am 5. April d. J. Vierteilung nach dem Symbol: 2 II 1.1.1.1 u. Von den neu entstandenen Individuen wurde das eine weiter kultiviert und man sah am 8. April d. J., daß es eine Teilung in zwei einkernige Nachkommen erfuhr. Von diesen wurde nur ein Nachkomme weiter gezüchtet, bis am 10. April d. J. abermals eine Zweiteilung und am 13. April eine folgende sich nachweisen ließ, deren Produkte insgesamt einkernig waren.

C. Ein zweikerniges, an Protoplasma durch Operation ärmeres Individuum wurde im Hohlgläschen vom 5. November 1898 an gezüchtet. Am 11. November d. J. Zweiteilung nach dem Symbol: 3 II 2.1. Die beiden gleich großen Nachkommen gerieten sofort wieder in Kultur. Am 14. November d. J. ließ sich konstatieren, daß der zweikernige Descendent eine Zweiteilung nach dem Schema: 1 II 1.1 erfuhr. Inzwischen wurde das einkernige Individuum zu einem zweikernigen und am 22. November sah man es bereits in drei Teile zerklüftet (Schema: 2 II 2.1.1). Der zweikernige Teil wurde weiter gezüchtet, jedoch am 20. Dezember ließ sich nichts mehr von ihm nachweisen, so daß man annehmen muß, daß ihn der Tod ereilt hat.

D. Ein zweikerniges, an Protoplasma ärmer gemachtes Individuum, seit 29. März 1900 im Uhrglas kultiviert, erscheint am 4. April d. J. nach dem Schema 2 II 1.1.1.1 geteilt.

E. Ein zweikerniges, an Plasmamasse durch Operation ärmeres Individuum wurde am 4. April 1900 im Uhrglas kultiviert. Am 7. April d. J. ließ sich eine stattgefundene Teilung nach dem Symbol: 2 II 2.1.1 wahrnehmen. Der zweikernige Nachkomme erschien am 9. April d. J. nach dem Symbol: 3 II 2.1 geteilt. Der erstere von beiden Nachkommen erfuhr eine Dreiteilung nach dem Symbol 2 II 2.1.1, wie am 12. April d. J. sich konstatieren ließ. Das zweikernige Tochterindividuum läßt schließlich am 13. April eine Zweiteilung wahrnehmen, die dem Schema: 1 II 1.1 angehört.

Schlußfolgerung. Die Versuchsresultate stimmen, wie man sieht, mit unsrer Voraussetzung gut überein. Die Individuen von *Amoeba proteus*, deren Protoplasma eine Reduktion erfuhr, haben sich nach denselben Regeln geteilt, wie die intakten Formen.

3. Versuche mit der sog. Lebendfärbung.

Werden mehrkernige Formen von *Amoeba proteus* intra vitam mit Hilfe stark verdünnter Lösungen von gewissen organischen Farbstoffen gefärbt, so läßt sich nachweisen, daß der Farbstoff an bestimmte, im Körper der Amöbe enthaltene Stoffe sich bindet, die ich als plasmabildende[1] bezeichnet habe.

Selbstverständlich können derartig auch die mehrkernigen Formen von *Amoeba proteus* behandelt werden, und es genügt, die letzteren nach deren Färbung nur in dieselben Lebensbedingungen zurückzubringen, denen die ungefärbten Individuen ausgesetzt waren.

Es steht nun die Frage offen, wie sich die Plasmateilung bezüglich deren Modifikationen gestalten wird, wenn künstlich gefärbte Individuen zu den Versuchen herangezogen werden.

In der Erwägung, daß die nachträgliche Aktion, derzufolge eine Amöbe sich von dem aufgenommenen Farbstoff zu befreien trachtet, mit einem Energieaufwand verknüpft sein dürfte, in der Erwägung ferner, daß, soviel sich urteilen läßt, trotz der Färbung die vitalen Funktionen intakt bleiben, ließ sich voraussetzen, daß es zu keinen andern Teilungsmodi bei den gefärbten Exemplaren als bei den ungefärbten Individuen kommen wird, sondern daß höchstens Differenzen in der Zeitfolge der successiven Teilungen bemerkbar sein werden.

Eine Lebendfärbung kann man schließlich noch damit kombinieren, daß man dem Protoplasten einen Teil seines Protoplasmas wegnimmt.

Was die Färbung der mehrkernigen Formen betrifft, so währte dieselbe volle 24 Stunden, wobei die oben genannten Zylindergläschen für die Aufnahme der verdünnten Farbstofflösungen samt den Amöben zur Anwendung kamen. Über das Nähere mag übrigens in meiner oben zitierten Arbeit nachgesehen werden. Es mögen schließlich die hierher gehörigen Versuche und deren Resultate kurz angeführt werden.

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[1] A. Štolc, Über das Verhalten des Neutralrots im lebendigen Protoplasma. Zeitschr. f. allgem. Physiol. 1902.

A. Ein zweikerniges, mit Vesuvin gefärbtes Individuum wurde seit 22. Juni 1898 im Uhrglas kultiviert. Am 26. Juni d. J. Dreiteilung nach dem Symbol: 2 II 2.1.1. Der zweikernige Nachkomme wurde dann weiter kultiviert. Am 27. Juni d. J. fand ich, daß er in vier einkernige Individuen zerfallen ist, von denen eines größer ist als die drei andern. Nur an diesem wird die Kultur fortgesetzt. Am 28. Juni d. J. erscheint es in zwei einkernige Nachkommen gespalten, von denen der eine weiter gezüchtet wurde. Am 29. Juni d. J. ergibt die Beobachtung eine Zweiteilung, deren Produkte einkernig waren.

B. Ein zweikerniges, mit Neutralrot gefärbtes und einer Plasmaportion beraubtes Individuum wurde am 26. März 1900 im Uhrglas in Kultur genommen. Am 28. März Zweiteilung nach der Formel: 1 II 1.1.

C. Ein zweikerniges, mit Neutralrot gefärbtes und einer Plasmaportion beraubtes Individuum wurde seit 26. März 1900 im Uhrglas gezüchtet. Am 3. April d. J. erschien der Körper geteilt, und zwar nach dem Symbol: 3 II 2.1. Der erstere, zweikernige Nachkomme wurde dann weiter gezüchtet, worauf am 4. April d. J. eine Dreiteilung nach dem Schema: 2 II 2.1.1 sich konstatieren ließ. Der zweikernige Descendent ergab schließlich am 8. April eine Vierteilung nach dem Symbol 2 II 1.1.1.1.

D. Ein zweikerniges, mit Neutralrot gefärbtes und einer Plasmaportion beraubtes Individuum wurde seit 5. April 1900 im Uhrglas der Kultur unterworfen. Am 6. April d. J. fand ich eine Dreiteilung nach dem Symbol: 2 II 2.1.1. Der zweikernige Nachkomme hat sich dann, nach der Beobachtung am 13. April d. J., weiter geteilt, indem die Teilung dem Typus: 1 II 1.1 entsprach. Beide neu entstandenen Tochterindividuen nahm ich abermals in Kultur. Am 15. April d. J. erhielt ich das einfachste Resultat einer Zweiteilung, wo nämlich die beiden Nachkommen einkernig waren.

E. Ein zweikerniges, mit Neutralrot gefärbtes und eines Protoplasmateils beraubtes Individuum wurde seit 6. April 1900 im Uhrglas gezüchtet. Am 9. April d. J. erhielt ich als Resultat eine Vierteilung nach dem Symbol: 2 II 1.1.1.1. Einer von den vier Nachkommen ergab in weiter fortgesetzter Kultur am 12. April d. J. eine Zweiteilung, deren Produkte einkernig waren.

Schlußfolgerung. Aus den hier angeführten Versuchsresultaten erhellt zur Genüge, daß der mehrkernige Protoplast (hier von *Amoeba* *proteus*) nach einer Lebendfärbung, eventuell nach dieser und einer nachfolgenden Abtrennung eines Protoplasmateils, zu keiner andern Teilung veranlaßt wird, die nicht auch bei den ungefärbten und intakten Exemplaren zutage getreten wäre.

Allgemeine Ergebnisse.

A. Mehrkerniges Protoplasma, wie es die mehrkernigen Formen von *Amoeba proteus* bieten, kann sich auf dreierlei Weise teilen:
   1) Das Protoplasma teilt sich, ohne daß gleichzeitig mit ihm die Kerne eine Teilung erführen.
   2) Das Protoplasma teilt sich und gleichzeitig mit ihm teilen sich alle Kerne.
   3) Das Protoplasma teilt sich, ohne daß gleichzeitig mit ihm sämtliche Kerne sich teilen würden.

B. Die Teilungsprodukte sind höchst mannigfaltig, besonders was ihre Anzahl, ihre Größe und die Zahl der in ihnen enthaltenen Kerne anbelangt.

C. Bei Anwendung gewisser Mittel, wie: 1) Einschränkung von Nährmaterial, 2) Wegnahme eines Protoplasmateils, 3) Lebendfärbung (durch Vesuvin), 4) Lebendfärbung und darauf folgende Wegnahme eines Protoplasmateils, finden keine andern Teilungsmodi statt.

Zum Schluß möge eine tabellarische Übersicht der ausgeführten Beobachtungen und Experimente beigefügt werden, worin sämtliche durch Symbole ausgedrückte Teilungsergebnisse enthalten sind.

1. **Abteilung** (Beobachtungen).

I. Teilung nach dem 1. Modus.

A. II, 31. Oktober 1898. Hohlgläschen.
3. November 1898 . . . . 1 II 1.1

B. II, 25. April 1900. Hohlgläschen und Uhrglas.
1. Mai 1900 . . . . 1 II 1.1

C. II, 21. Juni 1901. Uhrglas.
23. Juni 1901 . . . . 1 II 1.1 u

II. Teilung nach dem 2. Modus.

A. II, 24. April 1898. Uhrglas.
27. April 1898 . . . . 2 II 2.1.1

B. II, 10. Juni 1898. Uhrglas.
22. Juni 1898 . . . . 2 II 1.1.1.1 u

C.	II, 5. Oktober 1898. Uhrglas.	
	7. Oktober 1898	2 II 2.1.1
	9. Oktober 1898	2 II 1.1.1.1
D.	II, 12. April 1900. Uhrglas.	
	16. April 1900	2 II 1.1.1.1 u
E.	II, 20. April 1900. Uhrglas.	
	21. April 1900	2 II 2.1.1
	25. April 1900	2 II 2.1.1
	30. April 1900	2 II 2.1.1
	5. Mai 1900	2 II 1.1.1.1
F.	II, 21. April 1900. Uhrglas.	
	23. April 1900	2 II 1.1.1.1
G.	II, 22. April 1900. Hohlgläschen und Uhrglas.	
	24. April 1900	2 II 1.1.1.1
H.	II, 24. April 1900. Uhrglas.	
	25. April 1900	2 II 1.1.1.1
I.	II, 25. April 1900. Uhrglas.	
	27. April 1900	2 II 3.1
K.	II, 7. Mai 1900. Uhrglas.	
	9. Mai 1900	2 II 2.1. 1
L.	II, 29. September 1903. Zylindergläschen.	
	1. Oktober 1903	2 II 2.1.1
M.	II, 9. Oktober 1903. Zylindergläschen.	
	20. Oktober 1903	2 II 1.1.1.1
N.	II, 19. September 1903. Zylindergläschen.	
	23. September 1903	2 II 2.1.1 u
	29. September 1903	2 II 1.1.1.1 u

III. Teilung nach dem 3. Modus.

A.	II, 2. Mai 1900. Uhrglas.	
	4. Mai 1900	3 II 2.1
B.	II, 17. Oktober 1903. Zylindergläschen.	
	7. November 1903	3 II 1.1.1.
C.	IV, 28. September 1900. Uhrglas.	
	1. Oktober 1900	3 IV 3.1.1.1

IV. Teilung nach dem 1., 2. oder 3. Modus.

A.	III, 3. März 1897. Uhrglas.	
	11. März 1897	2 III 3.2.1
	13. März 1897	2 III 2.1.1.1.1 u
	16. März 1897	1 II 1.1
B.	II, 24. April 1898. Uhrglas.	
	27. April 1898	2 II 2.1.1
	30. April 1898	1 II 1.1
C.	II, 5. Oktober 1898. Hohlgläschen.	
	12. Oktober 1898	3 II 2.1
	13. Oktober 1898	3 II 2.1
	22. Oktober 1898	2 II 2.1.1
	26. Oktober 1898	2 II 2.2
	30. Oktober 1898	2 II 1.1.1.1

D. II, 3. September 1903. Zylindergläschen[1].

4. September 1903	2 II 2.1.1 u
8. September 1903	3 III 3.1.1 u
9. September 1903	2 III 3.1.2
10. September 1903	3 III 3.1
14. September 1903	2 II 2.1.1 u
28. September 1903	2 II 2.1.1
1. Oktober 1903	2 II 1.1.1.1
2. Oktober 1903	2 III 2.1.1.2
8. Oktober 1903	3 II 1.1.1
2. November 1903	3 IV 6.1

2. Abteilung (Versuche).

Versuch mit beschränkter Nahrung.

II, 5. November 1898. Uhrglas.

11. November 1898	3 II 2.1
30. November 1898	1 II 1.1

Versuche mit Abtrennung eines Protoplasmateils.

A. II, 24. Februar 1898. Uhrglas.

26. Februar 1898	2 II 2.1.1
2. März 1898	2 II 2.1.1
6. März 1898	2 II 1.1.1

B. II, 1. April 1898. Hohlglas.

5. April 1898	2 II 1.1.1.1 u

C. II, 5. November 1898. Hohlglas.

11. November 1898	3 II 2.1
14. November 1898	1 II 1.1
22. November 1898	2 II 2.1.1

D. II, 29. März 1900. Uhrglas.

4. April 1900	2 II 1.1.1.1

E. II, 4. April 1900. Uhrglas.

7. April 1900	2 II 2.1.1
9. April 1900	3 II 2.1
12. April 1900	2 II 2.1.1
13. April 1900	1 II 1.1

Versuch mit Vesuvin.

A. II, 22. Juni 1898. Uhrglas.

26. Juni 1898	2 II 2.1.1
27. Juni 1898	2 II 1.1.1.1 u

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[1] Descendenz durch vertikale punktierte Verbindungslinien angedeutet!

Versuche mit Neutralrot kombiniert mit Abtrennung eines Protoplasmateils.

B. II, 26. März 1900. Uhrglas.
28. März 1900 . . . . 1 II 1.1

C. II, 26. März 1900. Uhrglas.
3. April 1900 . . . . 3 II 2.1
4. April 1900 . . . . 2 II 2.1.1
8. April 1900 . . . . 2 II 1.1.1.1

D. II, 5. April 1900.
6. April 1900 . . . . 2 II 2.1.1
13. April 1900 . . . . 1 II 1.1

E. II, 6. April 1900.
9. April 1900 . . . . 2 II 1.1.1.1

lc, a.

Über kernlose Individuen und kernlose Teile von Amoeba proteus

Ein Beitrag zur Erforschung der plasmatischen und nucleären Tätigkeit

von

Antonín Štolc

Mit 2 Tafeln

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Über kernlose Individuen und kernlose Teile von Amoeba proteus.

Ein Beitrag zur Erforschung der plasmatischen und nucleären Tätigkeit.

Von

Antonín Štolc.

Mit Tafel IV und V.

Eingegangen am 8. Januar 1910.

Die Grundlagen dieser Arbeit bilden meine vom Jahre 1897 bis zum Jahre 1904 ausgeführten Beobachtungen, welche dann in der folgenden Zeit durch das Studium der Beziehungen zwischen dem Kern und dem Protoplasma ergänzt wurden.

I.

Es gelang mir zweimal, die Entstehung eines kernlosen Individuums von *Amoeba proteus* zu beobachten und seinen ferneren Lebenslauf zu verfolgen. Der erste Fall begegnete mir im Jahre 1903, der zweite im folgenden Jahre. Das erstemal handelte es sich um ein ursprünglich einkerniges Individuum, welches später zu einem zweikernigen wurde, um bei fortgesetzter isolierter Kultur nach einer Reihe von Tagen (am 8. September) in vier ungleiche Tochterindividuen sich zu teilen (Taf. IV Fig. 3, 4, 5, 6). Das eine Teilungsprodukt war groß, das andre klein, das dritte von mittlerer Größe, jedes von den dreien mit je einem Kern, einer kontraktilen Vacuole und mit ein wenig Nahrung; das vierte (Fig. 6) dagegen von einer mittleren Gestalt besaß zwar eine kontraktile Vacuole, führte etwas Nahrung, aber enthielt keinen Kern. Alle vier Individuen wurden dann in zwei cylindrischen Glasgefäßchen (von 7 cm Länge und mehr als 1 cm Breite) weiter gezüchtet, das große und kleine Individuum in einem Gläschen, das mittlere und kernlose im andern. Jeden Tag bekamen die Kulturen frisches Flußwasser nebst einer aus Infusorien bestehenden Nahrung, und täglich fand auch die Durchmusterung der lebenden Objekte statt. Am 13. September sah ich das große Individuum zweigeteilt, und nachdem ich den einen Nachkommen sofort isoliert in Kultur genommen hatte, konnte ich an ihm am 17. September wieder eine Zweiteilung konstatieren. Weitere Teilungen, wobei stets nur das eine Teilungsprodukt in Kultur genommen wurde, kamen am 23., 28. und 30. September sowie am 2. Oktober zur Beobachtung. Das mittlere Individuum fand ich am 14. September geteilt. Fernere Teilungen, wobei stets wieder nur das eine Teilungsprodukt kultiviert und beobachtet wurde, fanden am 27. September und am 1. Oktober statt. Das kleine Individuum nahm nur allmählich an Größe zu, bis am 30. September seine Zweiteilung konstatiert wurde, dessen Produkte untereinander und mit den Nachkommen des großen Individuums gleiche Größe besaßen. Das eine von den diesbezüglichen Teilungsprodukten nahm ich dann isoliert in Kultur und konnte am 3. Oktober seine Zweiteilung feststellen. Was das kernlose Individuum betrifft, so wurde nach mehreren Tagen (am 18. September) sein Tod konstatiert.

Den zweiten Fall, welcher die Entstehung eines kernlosen Individuums betrifft, habe ich in einer meiner früheren Arbeiten tangiert (siehe »Plasmodiogonie, eine Vermehrungsart der niedersten Protozoen«; im Archiv f. Entwicklungsmech. d. Organismen. 1906. S. 119 u. 120), worin über jene Formen von *Amoeba proteus* berichtet wird, welche den Übergang zwischen mehrkernigen Formen und jenen mit einem übermäßig großen Kern bilden. Am Ende des dort angeführten Beleges erwähne ich das Vorkommnis eines Individuums mit einem dreifachen Kern. Dieses Individuum wurde dann weiter gezüchtet, bis am 20. Juli seine Zweiteilung zutage trat. Hierbei entstanden zwei gleichgroße Individuen, das eine kernhaltig, das andre kernlos, beide mit je einer kontraktilen Vacuole. Das kernhaltige Individuum hat einen abnormalen, in zwei ungleich große Lappen eingeschnürten Kern.

Beide genannten Nachkommen wurden dann in einem Cylindergläschen weiter gezüchtet, wobei jeden Tag das Kulturwasser (Flußwasser) gegen frisches ausgetauscht und neue Nahrung hinzugefügt wurde. Täglich fand auch die Durchmusterung der betreffenden Objekte statt. Am 22. Juli fand ich, daß das kernhaltige Individuum eine Zweiteilung erfuhr, welche zur Entstehung eines größeren großkernigen und eines kleineren kleinkernigen Individuums führte. Am nächsten Tage konnte eine Zweiteilung des größeren Individuums konstatiert werden, welcher abermals ein größeres großkerniges und ein kleineres kleinkerniges Tochterindividuum entstammten. An dem alten kleinen und kleinkernigen Individuum wurde erst am 25. Juli eine Zweiteilung nachgewiesen, deren Produkte ebenfalls ungleich groß waren. Was das oben erwähnte kernlose Individuum betrifft, so hat sich dieses inzwischen natürlich nicht geteilt, bis am 24. Juli sein Tod registriert wurde (Taf. IV Fig. 16—22).

II.

Es möge hier weiter untersucht werden, wie sich die vitalen Tätigkeiten bei den kernlosen Individuen im Vergleich mit den kernhaltigen Exemplaren verhielten.

Es sei vorerst der Bewegungen gedacht. Normale, typische *Amoeba proteus*, wie sie z. B. Leidy[1]) in seinem großen Werke beschreibt und mit Abbildungen begleitet, zeigt während ihres Lebens zwei Zustände, welche durch bestimmte Körpergestalt und durch charakteristische Plasmabewegungen ausgezeichnet sind, nämlich: einen Gleichgewichts- und einen Reizzustand (Taf. IV Fig. 2 u. 1). Im ersteren Zustand kriecht *Amoeba proteus* über die Unterlage, indem sie der Nahrung nachgeht und dieselbe aufnimmt. Ihr Körper ist hierbei plattgedrückt, zur Unterlage angeschmiegt, einfach oder verzweigt. In seltenen Fällen gehen ihm distinkte Pseudopodien ab, so daß er eine limaxartige Gestalt besitzt; gewöhnlich führt er lappenartige oder fingerförmige Pseudopodien, welche entweder ebenfalls dem Substrat angeschmiegt sind, oder von diesem mehr oder weniger abstehen. In diesem Gleichgewichtszustand zeigt das Protoplasma zwei differente Bewegungsarten, und zwar eine Hauptbewegung oder fortschreitende Bewegung, welche von einer bestimmten Stelle des Körpers, die wir als hinteres Leibesende bezeichnen, ausgeht, und jene Nebenbewegungen, welche den Pseudopodien eigen sind.

Der Reizzustand entsteht aus dem Gleichgewichtszustand infolge eines physikalischen oder chemischen Reizimpulses. Nach einer gewissen Zeit, nachdem der Reiz überwunden wurde, kehrt *Amoeba proteus* wieder in ihren Gleichgewichtszustand zurück. Der Reizzustand ist leicht erhältlich und unter dem Mikroskop demonstrierbar, wenn man mechanisch eingreift, z. B. wenn man ein Exemplar von *Amoeba proteus* im Gleichgewichtszustand durch einen stärkeren, mittels einer Pipette erzielten Wasserstrom von dem als Unterlage dienenden Objektträger losreist. Im andern Falle wird der Reizzustand z. B. durch Temperaturänderung oder durch ein chemisches Mittel ausgelöst, indem man z. B. den Objektträger erwärmt, oder indem man eine Kochsalzpartikel im Wassertropfen, in welchem sich ein Individuum der *Amoeba proteus* befindet, löst. Solange der Reizzustand andauert, adhäriert *Amoeba proteus* nicht zur Unterlage, und am Körper ist ein Mittelteil unterscheidbar, von welchem nach verschiedenen Seiten strahlenartig fingerförmige Pseudopodien ausgehen.

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[1]) Leidy, Fresh-Water Rhizopods of North-America. (Washington 1879.)

Für den Reizzustand sind auch seine Plasmabewegungen charakteristisch, teils jene, welche in das Innere des Körpers einmünden (centripetale), teils centrifugale, welche die Entstehung von strahlenförmigen Pseudopodien veranlassen. Ich fand nun, daß auch den kernlosen Individuen beide Zustände eigen sind, wie den mit ihnen gleichzeitig entstandenen kernhaltigen Schwesterindividuen.

Der Gleichgewichtszustand konnte bei kernlosen Individuen bis zu jener Zeit verfolgt werden, wo deutliche Symptome des nahenden Absterbens zutage traten. Der Körper von kernlosen, dem Tode nahen Individuen zieht sich nämlich zusammen, adhäriert nicht mehr zur Unterlage, rundet sich allmählich mehr und mehr ab, wobei zusehends die Plasmabewegungen an Intensität abnehmen, indem z. B. die Pseudopodien nur auf niedrige, wellige Prominenzen sich beschränken, während im Körperinnern die Glanzkügelchen zusammentreten und Vacuolen erscheinen, als untrügliches Zeichen des kurz darauf folgenden Plasmazerfalls (vgl. Taf. IV Fig. 22).

Zum Beleg der hier angeführten Beobachtung sei hier das die beiden kernlosen Individuen betreffende Protokoll im Auszuge zitiert.

A. Das erste kernlose Individuum. Am 9. September: Es wurde kriechend an der Glaswand des zur Kultur dienenden Cylindergefäßchens vorgefunden. Nach seiner Abhebung zeigt es unter dem Mikroskop deutliche Merkmale des Reizzustandes. Allmählich aber kehrt es zum Gleichgewichtszustande zurück (Taf. IV Fig. 7 u. 8). Am 10. September: Der Körper des betreffenden Individuums adhäriert zum Substrat, es befindet sich im Zustande des Gleichgewichts, aus welchem es durch einen Reizimpuls in den Reizzustand übergeht. Am 11. September: Bei der Durchmusterung zeigt der Körper den typischen Reizzustand, welcher später dem normalen zum Substrat angedrückten Zustande wich. Am 13. September: Ich fand das Individuum an der Wand des Kulturgefäßchens kriechend von limaxartiger Gestalt. Abgehoben zeigt es unter dem Mikroskop einen schwachen Reizzustand und kehrt hier wieder zum limaxartigen Zustand zurück (Taf. IV Fig. 10—15). Am 15. September: Der Körper ist zusammengezogen, im Zustande schwacher Reizung und läßt im Innern das Zusammentreten von Glanzkügelchen hervortreten. Am 17. September ähnliche Erscheinungen wie am 15. September. Am 18. September: Es wurde der Tod des Individuums nachgewiesen.

B. Das zweite kernlose Individuum. Am 20. Juli: Es zeigt den zur Unterlage angeschmiegten Gleichgewichtszustand und geht durch Reizung in den Reizzustand über. Am 21. Juli: An dem Individuum läßt sich die Succession beider Zustände nachweisen, doch fangen im Körper die Glanzkügelchen an sich zu aggregieren (Taf. IV Fig. 16). Am 22. Juli: Das Individuum befindet sich noch im Gleichgewichtszustand, doch sind die Kügelchen einander schon stark genähert und es treten zwei große Vacuolen zutage (Taf. IV Fig. 18). Am 23. Juli: Das Individuum adhäriert nicht mehr zur Unterlage, zeigt noch deutlich den Reizzustand (Taf. IV Fig. 21), jedoch sein Körper neigt schon stark zur kugligen Gestalt und führt in seinem Innern einen Haufen von Glanzkügelchen nebst einer großen Vacuole. Am 24. Juli. Es wurde der Tod des Individuums konstatiert. Es mag noch hinzugefügt werden, daß bei den beiden kernlosen Individuen, von denen oben die Rede war, die kontraktile Vacuole — sofern nicht schon die besagten Anzeichen des nahen Todes zu Gesicht kamen — sich in ihren Pulsationen ebenso verhielt wie bei den kernhaltigen Exemplaren.

Bei den beiden kernlosen Individuen, von denen eben berichtet wurde, führte schon bei deren Entstehung das Protoplasma verschiedene Nahrungspartikelchen. Neue Nahrung wurde jedoch, wie direkte Beobachtung ergab, von den kernlosen Individuen nicht aufgenommen, während eine solche Aufnahme bei den kernhaltigen Schwesterprodukten häufig stattfand.

III.

Hans Prandtl beschreibt in seiner über die physiologische Degeneration von *Amoeba proteus* handelnden Arbeit[1]) die Entstehung von kernlosen Individuen infolge einer Degeneration des

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[1]) Hans Prandtl, Die physiologische Degeneration der *Amoeba proteus*. Archiv f. Protistenkunde. 1907.

Kerns. Die von Prandtl beobachtete physiologische Degeneration ist jedoch keine normal physiologische, sondern jedenfalls eine pathologische Erscheinung. In seiner Studie berührt auch Prandtl einige meiner Ausführungen, welche über die Plasmodiogonie als eine besondere Vermehrungsart der niedrigsten Protozoen handeln. Ihm scheint die Definition der Plasmodiogonie unklar zu sein. Wer meine Arbeit ein wenig aufmerksamer gelesen hat, kann darüber nicht im Zweifel sein, was ich unter dem Begriff Plasmodiogonie verstehe, nämlich: Die Entstehung eines mehrkernigen Zustandes entweder durch Kernteilung eines ursprünglich einkernigen Individuums oder durch ein nur auf das Plasma beschränktes Verschmelzen von einkernigen Individuen, welcher Zustand nach einiger Zeit zur Entstehung einkerniger Individuen führt. Vom Standpunkte der Phylogenie ist natürlich die Plasmodiogonie eine »alte Erscheinung«, ich aber habe das erstemal getrachtet, auf sie als auf einen besonderen Modus der Vermehrung niederster Protozoen aufmerksam zu machen. Der nicht voreingenommene Leser wird dann ebenfalls meine aus der Plasmodiogonie sich ergebenden Deduktionen nicht als sonderbar finden, wodurch dieselbe als ein Mittel zur größeren Komplikation des Plasmas und Variabilität der neuen aus mehrkernigen Zuständen hervorgegangenen Individuen angesehen wird.

IV.

Mit Rücksicht auf das hier dargestellte Verhalten der kernlosen Individuen von *Amoeba proteus* möge in dem folgenden noch das Verhalten von künstlich hergestellten kernlosen Stücken von *Amoeba proteus* einer eingehenden Untersuchung unterzogen werden. Die diesbezüglichen Individuen lassen sich leicht mittels einer scharfen Nadel in ein kernhaltiges und ein kernloses Stück zerlegen. In seiner verdienstvollen Arbeit hat bereits Hofer[1]) eine größere Anzahl von Experimenten mit künstlich hergestellten kernlosen Stücken von *Amoeba proteus* ausgeführt, um den Einfluß des Kerns auf das Protoplasma zu studieren.

Kernhaltige und kernlose Teile von *Amoeba proteus* haben mir auch schon einmal das Material abgegeben, und zwar für meine Arbeit[2]), welche über das Verhalten des Neutralrots im lebendigen Plasma handelt.

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[1]) Bruno Hofer, Experimentelle Untersuchungen über den Einfluß des Kerns auf das Protoplasma. Jenaische Zeitschr. f. Naturw. 1890.

[2]) Antonín Štolc, Über das Verhalten des Neutralrots im lebendigen Protoplasma. Zeitschr. f. allgem. Physiolog. 1902.

Kernlose Teile wurden gemeinsam mit den kernhaltigen entweder auf einem in der Mitte konkav ausgeschliffenen Objektträger oder im Uhrgläschen oder aber in cylindrischen Glasgefäßchen von angegeben Dimensionen gezüchtet. Täglich tauschte ich das Kulturwasser (Flußwasser) gegen frisches aus und setzte neue Nahrung hinzu, wobei ich täglich alle Versuchsobjekte durchmusterte. Die kernhaltigen Teile wachsen und teilen sich wiederholt, während die kernlosen nach einer kürzeren oder längeren Zeit zugrunde gehen. Es gelang mir hierbei, die kernlosen Teile im Mittel eine längere Zeit am Leben zu erhalten, als es HOFER gelang. In den von HOFER registrierten Fällen lebten die kernlosen Teile im Mittel 9—10 Tage, in einigen von mir untersuchten Fällen lebten sie mehr als zweimal, ja sogar dreimal so lang (mehr als 20 und 30 Tage).

V.

Was die locomotorische Tätigkeit der kernlosen Teile im Vergleich zu den kernhaltigen betrifft, so gelangt HOFER zu dem Resultat, daß die kernlosen Teile zwar plasmatische Bewegungen äußern, aber daß letztere unregelmäßig und sozusagen nicht geordnet sind im Vergleich zu jenen Bewegungen, welche den kernhaltigen Teilen oder intakten Individuen zukommen. Es deduziert daraus infolgedessen HOFER den Satz, daß der Kern einen die Plasmabewegungen ordnenden Einfluß ausübt, daß er in dieser Hinsicht als ein Regulationscentrum angesehen werden muß.

Resultate meiner an kernlosen Teilen von *Amoeba proteus* ausgeführten Beobachtungen stimmen mit den Ergebnissen der Beobachtungen von VERWORN[1]) und einiger andrer Forscher überein, denen gemäß kernlose Köperteile von Protozoen dieselben charakteristischen Bewegungen äußern können, welche intakten Individuen zukommen. An einem womöglich tadellosen Material, welches niemals durch Operation geschädigt wurde und auch sonst durch keinen nachteiligen Einfluß des Milieus gelitten hat, gelang mir der Nachweis, daß auch den kernlosen Körperteilen von *Amoeba proteus* die beiden Hauptzustände, welche durch besondere Körperformen und durch besondere Plasmabewegungen charakterisiert sind, zukommen, und daß hierin kein Unterschied von den kernhaltigen Körperteilen oder intakten kernhaltigen Individuen besteht.

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[1]) MAX VERWORN, Psychophysiologische Protistenstudien. Experimentelle Untersuchungen. Jena 1889. — Allgemeine Physiologie. Jena 1909. 5. Auflage. S. 623 u. f.

Der Gleichgewichtszustand kommt bei kernlosen Teilen bis zu jener Zeit vor, wo schon die Anzeichen des Absterbens sich einstellen; der Reizzustand aber auch in dieser morbiden Periode, um jedoch allmählich mit dem Tode auszuklingen. Die Symptome des Absterbens weichen im übrigen von jenen, welche bei kernlosen Individuen vorkommen, in keiner Beziehung ab. Der Körper wird allmählich starrer, indem er Kugelform annimmt, die Bewegungen nehmen an Intensität zusehends ab, beschränken sich auf geringe peripherische Umrißänderungen; im Innern treten die daselbst befindlichen Glanzkügelchen zusammen und es erscheinen Vacuolen als Zeichen des Plasmazerfalls (Taf. V Fig. 12, 13).

In bezug auf die locomotorische Tätigkeit der kernlosen Teile von *Amoeba proteus* seien hier in der Kürze einige Belege der Beobachtungsprotokolle angeführt.

1) Ein Individuum wurde (am 19. Februar 1898) in einen kernhaltigen und einen kernfreien Teil gespalten. Beide Teile wurden gemeinsam am Hohlgläschen gezüchtet. Am 28. Februar erscheint der kernfreie Teil plattgedrückt, er adhäriert zu dem Substrat, kriecht und besitzt eine limaxartige Gestalt. Am 15. März wurde sein Tod konstatiert.

2) Ein Individuum wurde (am 3. Februar 1898) künstlich in einen kernhaltigen und einen kernlosen Teil gespalten. Beide Objekte wurden gemeinsam im Uhrgläschen gezüchtet. Am 14. Februar ergab die Beobachtung anfangs einen ausgeprägten Reizzustand, welcher alsbald in den Gleichgewichtszustand überging, indem der Körper platter wurde und wieder an das Substrat sich anschmiegte. Am 21. Februar wurde der Tod konstatiert.

3) Ein Individuum wurde (am 25. Dezember 1899) in einen kernhaltigen und einen kernlosen Teil zerspalten. Beide Teile wurden im Hohlgläschen gemeinsam weiter gezüchtet. Der kernhaltige Teil hat sich am 31. Dezember in zwei Individuen geteilt, von welchen nur das eine weiter gezüchtet wurde, bis es am 6. Januar 1900 abermals eine Teilung zeigte. Der kernlose Teil weist bei der am 6. Januar stattgefundenen Durchmusterung dieselben Plasmabewegungen auf wie das mit ihm gleichzeitig kultivierte Individum. Die beiden Hauptzustände lassen sich an ihm beobachten (Taf. V Fig. 9, 10). Im Gleichgewichtszustande ist der Körper plattgedrückt, kriecht am Substrat hinweg und ist demselben angeschmiegt. Sollte er losgetrennt werden, mußte ein Wasserstrom mittels der Pipette auf ihn gerichtet werden.

Aus den angeführten Belegen erhellt zur Genüge, daß der Kern keinen unmittelbaren ordnenden Einfluß auf die Plasmabewegungen ausübt. Außerdem lehrt die lang anhaltende Fähigkeit der kernlosen Teile zur Bildung des Gleichgewichtszustandes, daß von einer Nachwirkung des Kerns kaum die Rede sein kann.

VI.

Es ist bekannt, daß kernlose Körperteile der Protozoen einer Neubildung der kontraktilen Vacuole fähig sind. Letztere hat man sich gewöhnt als ein Excretionsorgan zu betrachten, welches verschiedene Stoffe, und auch die im Atmungsprozeß entstandene Kohlensäure, nach außen befördert. Auch Hofer hat die Neubildung und Tätigkeit der kontraktilen Vacuole bei kernlosen Teilen von *Amoeba proteus* beobachtet. Seine Beobachtungen konnte ich an dem mir zur Verfügung gestandenen Material bestätigen. Anfänglich pulsiert die kontraktile Vacuole bei den kernfreien Teilen ebenso rasch wie bei den kernhaltigen oder bei den intakten Individuen. Erst als die ersten Anzeichen des nahenden Todes wahrgenommen werden, sind die Pausen zwischen der Diastole und Systole immer beträchtlicher. Es möge hier kurz über die Entstehung der kontraktilen Vacuole, wie ich sie in einem Falle bei einem kernlosen Teile detaillierter verfolgt habe, berichtet werden. Die kontraktile Vacuole, nachdem sie einen gewissen Umfang erreicht hat, zieht sich zusammen. Es erscheinen nacheinander drei kleine Vacuolen; zwei verschmelzen miteinander zuerst, dann verschmilzt auch die dritte Vacuole, wodurch eine größere Vacuole sich bildet, welche sich vergrößert, um nach einigen Minuten sich zu kontrahieren.

VII.

Hofer gelang es nicht, bei kernlosen Teilen von *Amoeba proteus* eine Nahrungsaufnahme zu konstatieren. Alle Versuche, welche darauf hinausgingen, diese Teile unter verschiedenen Bedingungen zu ernähren, blieben erfolglos, während die kernhaltigen Teile ihre Aufnahmefähigkeit stets zeigten. Demgegenüber gelang mir der Nachweis der Nahrungsaufnahme bei kernfreien Teilen zu wiederholten Malen, ja sogar in sehr zahlreichen Fällen. Was das Formelle des Vorgangs betrifft, so besteht kein Unterschied zwischen den kernhaltigen und kernfreien Teilen, sowie den intakten Individuen. Das Protoplasma sendet der Beute Plasmaströme entgegen, welche dieselbe umzingeln und darob verschmelzen, so daß die Beute in die

Aufnahmevacuole gelangt. Letztere zieht sich dann immer mehr und mehr zusammen, bis die Beute vom Protoplasma dicht umschlungen erscheint und in diesem getötet wird.

Die Beobachtungen, welche die Aufnahme von Nahrungskörper seitens der kernlosen Teile der *Amoeba proteus* betreffen, halte ich für so überaus wichtig, daß ich nicht umhin kann, den Protokollen detaillierte Belege zu entnehmen und hier folgen zu lassen.

1) Ein Individuum, welches keine Nahrung führte, wurde in einen kernhaltigen und einen kernfreien Teil gespalten. Beide Teile kultivierte ich gemeinsam in einem Uhrgläschen, wobei täglich das Flußwasser gegen frisches ausgetauscht und neue Nahrung, welche aus Ciliaten und Flagellaten bestand, hinzugefügt wurde. Alle 24 Stunden fand die Durchmusterung beider Objekte statt. Am zweiten Tage fand ich bei dem kernhaltigen Teile im Protoplasma eine Menge Nahrung, welche zum Teil schon zerstört war, teils aber aus frisch gefangenen Individuen von *Coleps* und *Chilomonas* bestand. Der kernfreie Teil enthielt im Plasma ebenfalls Nahrungsbissen, und zwar ein frisch gefangenes *Chilomonas*-Individuum, welches noch Bewegungen zeigte, dann ein ähnliches schon früher eingeführtes und unbewegliches Tier, und schließlich noch ein drittes, welches aber schon zerstört und in der Verdauungsvacuole sich befand (Taf. V Fig. 11).

2) Ein Individuum wurde (am 3. Februar 1898) in einen kernhaltigen und einen kernlosen Teil gespalten. Beide Teile wurden im Uhrgläschen in frischem Wasser nach Hinzufügung von reichlicher Nahrung (Ciliaten und Flagellaten) weiter gezüchtet. Am 5. Februar sah ich, daß der kernlose Teil bereits frische Nahrung aufgenommen hatte, und zwar in Gestalt eines Infusoriums, welches in der Aufnahmevacuole noch Bewegungen zeigte. Am 7. Februar konnte ich abermals eine Nahrungsaufnahme seitens desselben kernfreien Stückes nachweisen, da ein Infusorium in der Aufnahmevacuole sich befand und darin sich bewegte. Am 11. Februar wurde abermals die Aufnahme eines Ciliaten festgestellt.

3) Ein Individuum wurde (am 9. März 1897) in einen kernhaltigen und einen kernfreien Teil gespalten, welch beide Teile dann auf einem Hohlgläschen im frischen Wasser bei Gegenwart von geeigneter Nahrung (Ciliaten und Flagellaten) gemeinsam weiter gezüchtet wurden. Der kernfreie Teil hat noch am selben Tage ein ciliates Infusorium gefangen, welches in der Empfangsvacuole sich lebhaft bewegte. Am 13. März wurden in eine Aufnahmevacuole

zwei Flagellaten gefangen (Genus *Chilomonas*), von welchen das eine heftiger bewegliche Individuum aus der Vacuole sich befreite, während von dem andern sich das Protoplasma dann zurückzog. Am 14. März sah ich, daß der kernfreie Teil soeben ein Flagellatenindividuum in die Empfangsvacuole aufgenommen hat (Taf. V Fig. 4, 5); nach zwei Stunden hat sich dann die Empfangsvacuole zusammengezogen und die Beute war dicht vom Protoplasma umschlungen.

4) Ein fast nahrungsfreies Individuum wurde (am 23. März 1897) in einen kernhaltigen und einen kernfreien Teil zerlegt. Beide Teile habe ich dann am Hohlgläschen in frischem Wasser unter steter Nahrungserneuerung (ciliate und flagellate Infusorien) gemeinsam weiter gezüchtet. Am 24. März zeigt sich, daß der kernhaltige Teil im Protoplasma reichlich Nahrung aufgenommen hat (Taf. V Fig. 3). Dagegen hat der kernfreie Teil kurz vor der 11. Stunde vormittags ein Flagellatenindividuum (Genus *Chilomonas*) in eine geräumige Empfangsvacuole aufgenommen, worin die Beute abwechselnd heftig sich bewegt und wieder zur Ruhe kommt (Taf. V Fig. 1). Bis zur 12. Stunde wurden noch zwei andre lebhaft bewegliche Flagellaten (derselben Gattung) gefangen, von welchen später das eine Individuum aus der Vacuole entwich (Taf. V Fig. 2). Am 25. März findet sich im Protoplasma des kernfreien Teiles ein zerstörtes Individuum der Gattung *Coleps* und eine einzellige Grünalge.

5) Ein kernloses Stück wurde (vom 1. April 1897) auf einem Hohlgläschen gezüchtet. Bei der Durchmusterung vom 2. April wurde im Protoplasma aufgenommene Nahrung konstatiert (einige Individuen der Flagellatengattung *Chilomonas*, ein Individuum der Ciliatengattung *Glaucoma*, eine grüne Alge; Taf. V Fig. 6). Weiter hat man beobachtet, daß soeben ein Individuum der Gattung *Glaucoma* gefangen wurde. Der plasmatische Strom hat den Körper des Infusoriums in solcher Weise umschlungen, daß ein Teil desselben außerhalb des Protoplasmas blieb, indem er mit dem andern (größeren) von der Empfangsvacuole umgebenen Teile durch eine stielförmige Einengung verbunden wurde (Taf. V Fig. 6). Im folgenden Augenblick wurde diese Einengung durchgerissen, der größere Teil des Infusoriums wurde vollkommen von der Empfangsvacuole umgeben, dagegen blieb der kleinere Teil definitiv in einer Kugelform außerhalb des kernlosen Stückes (Taf. V Fig. 7). In den folgenden Tagen fand man im Protoplasma des kernlosen Stückes glänzende Nahrungsballen, bis am 5. April keine Spur von Nahrung aufgefunden werden konnte. Am 12. April wurde der Tod des kernlosen Stückes konstatiert.

6) Ein mittels Neutralrots in vivo gefärbtes Individuum wurde (am 25. März 1900) in einen kernhaltigen und einen kernfreien Teil zerlegt. Beide Teilstücke gerieten zum Zwecke weiterer gemeinsamer Kultur in ein Cylindergläschen mit frischem Flußwasser und frischer Nahrung (Infusorien). Am 28. März hat der kernhaltige Teil in seinem Innern reichliche frische Nahrung. Am 2. April fand ich im Protoplasma des kernfreien Teiles ein frisch gefangenes Individuum der Ciliatengattung *Coleps.* Am 5. April ergab die Durchmusterung, daß der kernfreie Teil soeben in eine große Empfangsvacuole ein Ciliatenindividuum der Gattung *Lembadion* gefangen hat. Am 18. April war der kernfreie Teil nicht mehr am Leben.

7) Zwei in vivo mittels Neutralrots gefärbte Individuen wurden am 26. Februar 1900) in je einen kernhaltigen und je einen kernlosen Teil zerlegt. Alle vier Teile wurden gemeinsam auf einem Hohlgläschen weiter gezüchtet, indem sowohl das Wasser als auch die Nahrung häufig erneuert wurden. Einer von den beiden kernlosen Teilen ging kurz darauf zugrunde, während der andre länger lebte. Am 11. März wurde er besichtigt, und nachdem wieder neue Nahrung und frisches Wasser hinzugefügt wurde, ergibt eine abermalige Beobachtung, daß sich das kernlose Stück im Zustande des Gleichgewichts befindet und soeben ein Ciliatenindividuum der Gattung *Coleps* gefangen hat. Am 22. März war von dem kernlosen Teile keine Spur mehr vorhanden.

VIII.

Die Nahrungsaufnahme bei den Individuen der *Amoeba proteus* geschieht an beliebiger Körperstelle. Der Nahrungsbissen wird in eine Empfangsvacuole (Aufnahmevacuole) eingeschlossen, welch letztere sich später zusammenzieht, bis die Beute vom Protoplasma dicht umschlossen wird.

Stets gerät jedoch die Nahrung infolge der Plasmaströmungen schließlich ans Hinterende des Körpers, wo sie eine bestimmte Zeit verharrt. Sind die Dimensionen der Nahrungsbissen größer, so werden letztere auf kleinere Partikel zerbröckelt. Es greift dann sofort die chemisch-physiologische Bearbeitung des eingeführten Nahrungsmaterials ein. Um die Nahrungsbissen herum zeigt sich ein flüssiger Hof, es entsteht eben dann die Verdauungsvacuole, worin die Nahrung verdaut wird. Die in der Vacuole der Verdauung unterworfene Nahrung wird durch den plasmatischen Hauptstrom, welcher am Hinterende des Amöbenkörpers beginnt, mitgerissen und wird zum Vorderende geführt. Hier taucht sie an der Oberfläche empor, gelangt zum Hinterende, gerät wieder in den Hauptstrom, um abermals zum Vorderende des Körpers sich zu bewegen usw. Während dieser Circulation wird die Nahrung verdaut, die eventuellen nicht mehr verdaulichen Reste werden dann am hinteren Körperteile ausgegeben. In meiner Arbeit, welche von dem Verhalten des Neutralrots im lebendigen Körper der *Amoeba proteus* berichtet, zeigte ich, daß die Verarbeitung der eingeführten eiweißhaltigen Nahrung so stattfindet, daß diese bis auf Aminosäuren sich zerlegt, was durch das Auftreten von Leucinkriställchen in den Verdauungsvacuolen bezeugt wird.

Hat man mittels Neutralrots eine Lebendfärbung des Körpers von *Amoeba proteus* vorgenommen, so sieht man, wie ich in der besagten Arbeit gezeigt habe, daß sich sowohl die Nahrungsballen als auch der flüssige Inhalt der Nahrungsvacuolen (Verdauungsvacuolen) und derjenige der mit Leucinkriställchen gefüllten kleinen Vacuolen rot färbt. Bei behutsamer Färbung, um nicht die vitalen Tätigkeiten zu alterieren, findet man an dem hinteren Körperende ein besonderes Verhalten. Man entdeckt nämlich eine Gruppe feiner mit einem Stich ins Violette intensiv rot gefärbter Körnchen.

Diese Körnchen sind in einen Plasmakörper von undeutlichen Umrissen eingebettet (Taf. V Fig. 14, *z*). Weil nun die Verdauung der aufgenommenen Nahrung am Hinterleibe beginnt, so könnte man die Anwesenheit der besagten Körnchen in eine Causalbeziehung mit der Verdauung bringen, indem man sie etwa als ein Proferment ansieht, aus welchem dann ein Ferment entsteht, welches in einer flüssigen Form die aufgenommene Nahrung verdaut, um so zur Entstehung der Verdauungsvacuolen den Anlaß zu geben. Den die Körnchen enthaltenden plasmatischen Körper kann man auch mit der Existenz des plasmatischen Hauptstromes, welcher vom Hinterende der *Amoeba* den Anfang nimmt und zum entgegengesetzten Körperende sich fortsetzt, in Zusammenhang bringen.

Man kann mit der größten Wahrscheinlichkeit dafürhalten, daß der plasmatische mit Profermentkörnchen gefüllte Körper durch seine chemische Natur den Impuls zu dem genannten Strömen gibt. Das Protoplasma, welches diesen Körper berührt, »zieht sich zusammen«. Es wird von der Körperoberfläche ins Innere getrieben, von hier aus drängt es sich nach vorn, wodurch der plasmatische Hauptstrom in dem Amöbenkörper realisiert wird. Ich vermute, daß ich nicht irre, wenn ich auf Grund der angeführten Umstände jenen plasmatischen Körper, welcher die vermutlichen Profermentkörnchen (von ausgeprägt chemischem Charakter) enthält, für ein Homologon des Centrosoms halte.

IX.

Hofer, welcher bei seinen zum Zwecke der Erforschung der Verdauung bei *Amoeba proteus* ausgeführten Untersuchungen Lebendfärbungen mittels des Bismarckbrauns (Vesuvins) ausgeführt hat, kam zu dem Resultat, daß die in Verdauung begriffene Nahrung Schritt für Schritt intensiver sich färbt als die der Verdauung noch nicht anheimgefallene. Er fand ferner, daß kernlose Körperteile von *Amoeba proteus* nur insofern der Verdauung fähig sind, als ihnen schon während der Teilung eine genügende Menge des Verdauungssecretes (Enzyms) zuteil wurde. Nach Hofer geht den kernlosen Körperteilen der *Amoeba proteus* die Fähigkeit, Verdauungssecrete zu produzieren, vollständig ab, und es ist nach ihm diese Tätigkeit unbedingt an die Mitwirkung des Kerns gebunden. Auf Grund eines umfangreichen Materials, welches ich in dieser Richtung untersuchen konnte, fand ich, daß die kernlosen Teile der *Amoeba proteus* nicht nur der Nahrungsaufnahme fähig sind, sondern daß dieselben auch die aufgenommene Nahrung zu verdauen vermögen, was in gleicher Weise von der anfangs als auch in späteren Lebensphasen aufgenommenen Nahrung gilt.

Es mögen hier noch eingehendere Belege folgen. 1) Der für die Nahrungsaufnahme angeführte Beleg Nr. 1 ist gleichzeitig auch ein Beleg für die Nahrungsverdauung. Das durch künstliche Teilung eines völlig nahrungsfreien Individuums entstandene kernfreie Stück hat im Verlaufe von 24 Stunden drei Exemplare der Flagellaten *Chilomonas* aufgenommen. Das eine vom Plasma dicht umschlungene Exemplar war frisch gefangen und somit noch beweglich, das andre ebenfalls vom Plasma umgebene Exemplar äußert keine Bewegung mehr, das dritte dagegen befindet sich im Stadium der Zerstörung und wird von einer Verdauungsvacuole umgeben.

2) Ein kernfreies Stück wurde (vom 1. April 1897) am Hohlgläschen kultiviert. Am 2. April zeigt die Durchmusterung im Protoplasma eingeführte Nahrung. Es ist zu unterscheiden, daß diese aus Infusorien und einer größeren einzelligen Grünalge besteht. Am 3. April befinden sich im Plasma runde Körper, nämlich Nahrungsballen, und ähnlich auch am nächstfolgenden Tage. Außerdem läßt sich eine

Nahrungsvacuole wahrnehmen, in welcher eine Gruppe von braunen aus der zerstörten Grünalge entstandenen Körperchen liegt. Am 5. April wurde gefunden, daß im Protoplasma weder Nahrungsballen noch die besagte Gruppe von braunen Körperchen sich vorfindet. In den folgenden Tagen fand keine Nahrungsaufnahme mehr statt, bis am 12. April der Tod des kernlosen Teiles konstatiert wurde.

3) Der als Beleg für Nahrungsaufnahme sub 4 angeführte Fall wurde weiterer Beobachtung unterworfen. Am 26. März fand sich im Plasma noch ein Überrest des zerstörten Colepsindividuums und ein aus der grünen Alge entstandenes braunes Zerstörungsprodukt. Am 27. März wurde der Überrest des Infusoriums nicht mehr angetroffen, wohl aber noch der dunkelbraune, etwas zusammengesunkene Körper. Am 28. März ist letzterer nicht mehr im Plasma anzutreffen.

4) Ein Individuum wurde der Lebendfärbung durch Methylenblau unterworfen. Es zeigt nach seiner Färbung die Nahrungsballen intensiv blau gefärbt. Jetzt wurde das Individuum (am 27. Februar 1900) in einen kernhaltigen und einen kernlosen Teil zerlegt, welch beide Teile dann einer gemeinsamen Kultur in frischem Wasser nebst Nahrungszusatz (in einem Uhrgläschen) unterworfen wurden. Am 3. März erscheint der kernhaltige Teil in zwei kernhaltige Individuen geteilt. Der kernlose Teil führt im Plasma kleine Nahrungsballen, welche tief blau gefärbt erscheinen. Von den kernhaltigen Individuen wurde nur das eine weiter gezüchtet. Am 9. März fand man dasselbe zweigeteilt. Der kernlose Teil, welcher am selben Tage durchmustert wurde, zeigt im Plasma kleine noch lebhaft blau gefärbte Nahrungsballen, daneben führt er aber auch einen soeben gefangenen *Coleps*. Am folgenden Tage ist der Körper der Beute bereits zerstört und blau gefärbt. Im Protoplasma befinden sich dann außerdem kleine blau gefärbte Nahrungsballen. Am 11. März wurde nur noch ein unbedeutender gefärbter Überrest des Infusoriums vorgefunden, während im Protoplasma daneben noch kleine blaue Nahrungsballen zur Beobachtung kamen. Am 13. März zeigt der kernlose Teil nur kleine blau gefärbte Nahrungsballen.

X.

Kernfreie Körperteile von *Amoeba proteus*, welche die Nahrung bereits aufgenommen und verdaut haben, neue Nahrung indessen nicht mehr zu sich nahmen, zeigen am Ende dieses Aktes im Protoplasma keine Nahrungsüberreste, sondern nur Leucinkriställchen, welche nach meiner früheren Beweisführung als eins der letzten

Verdauungsprodukte zu betrachten sind. Diese Kriställchen nehmen bis zum Tode der kernlosen Amöbenteile an Größe fortwährend zu. Danach muß man schließen, daß dem kernlosen Plasma die Fähigkeit abgeht, diese Kriställchen in einer Richtung zu benutzen, d. h. aus ihnen und noch andern Aminosäuren das lebendige, den lebenden Körper zusammensetzende Eiweiß zu konstruieren. Nur unter der Mitwirkung des Kerns besitzt das Plasma das Vermögen, die lebendige Substanz aufzubauen. Die kernlosen Körperteile der *Amoeba proteus*, da sie Nahrung aufnehmen und verdauen können, haben eine genügende Energiequelle, könnten somit sehr lange am Leben bleiben, wenn schließlich die Lebensmaschine sich nicht abnützen würde. Die Anhäufung von Stoffen im Protoplasma, welche nicht mehr verbraucht werden können, übt einen schädlichen Einfluß aus. Produkte der Eiweißverdauung, welche das Protoplasma nicht beseitigen kann, indem ihm die Fähigkeit abgeht, daraus lebendige Substanz aufzubauen, sofern sie ein bestimmtes Maß der Anhäufung überschreiten, beeinflussen das Protoplasma schädlich und haben nach gewisser Zeit seinen Tod zur Folge.

Aus dem Studium der kernlosen Individuen und der kernlosen Teilstücke der *Amoeba proteus* ergeben sich, was die Tätigkeit des Plasmas und des Kerns betrifft, folgende Schlüsse:

1) Das kernlose Plasma ist einer durch bestimmte Bewegungsreaktionen sich äußernde Reizbarkeit fähig.

2) Das kernlose Plasma führt dieselben charakteristischen Bewegungen aus wie das kernhaltige Protoplasma.

3) Das kernlose Plasma ist einer Atmungs- und Excretionstätigkeit fähig.

4) Das kernlose Plasma vermag Nahrung aufzunehmen und selbe zu verdauen.

5) Das kernlose Plasma ist einer Assimilation in der Richtung der Konstruktion des lebendigen Eiweißstoffes (der lebendigen Substanz) nicht fähig. Das vermag das Plasma nur unter der Mitwirkung des Kerns.

Erklärung der Abbildungen.

Buchstabenerklärung.

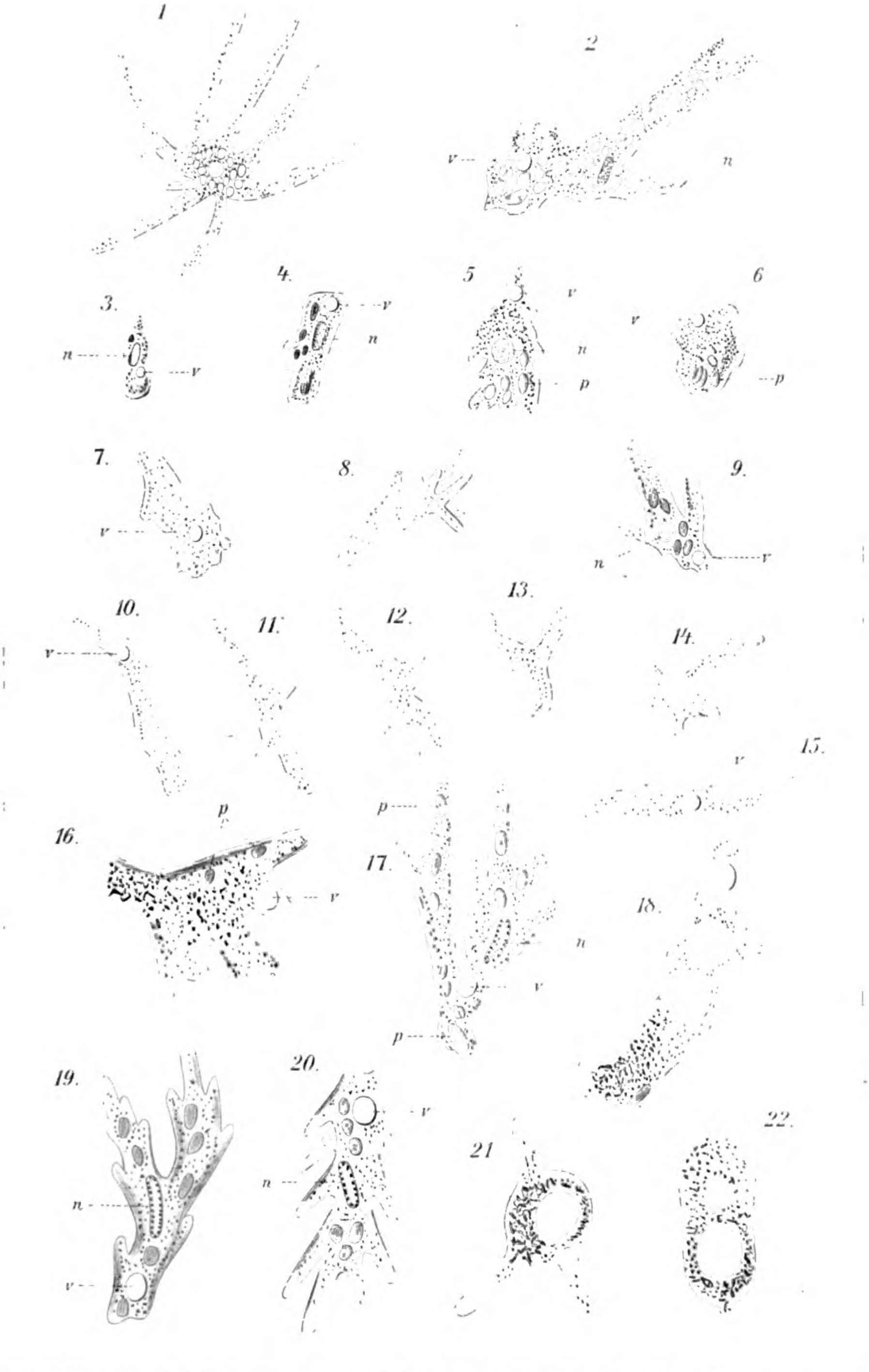
n Kern, *v* kontraktile Vacuole, *p* Nahrung, *pv* Empfangsvacuole,
tv Verdauungsvacuole.

Tafel IV.

Fig. 1. Der Reizzustand.
Fig. 2. Der Gleichgewichtszustand.
Fig. 3, 4, 5. Kernhaltige Individuen.
Fig. 6. Ein kernloses Individuum.
Fig. 7, 8. Ein kernloses Individuum im Zustand des Gleichgewichts und der Reizung.
Fig. 9. Ein kernhaltiges Individuum im Zustand des Gleichgewichts.
Fig. 10—15. Ein kernloses Individuum, welches aus dem Zustand des Gleichgewichts in jenen der Reizung und dann abermals in denjenigen des Gleichgewichts übergeht.
Fig. 16. Ein kernloses Individuum im Zustand des Gleichgewichts.
Fig. 17. Ein kernhaltiges Individuum im Zustand des Gleichgewichts.
Fig. 18. Ein kernloses Individuum im Zustand des Gleichgewichts.
Fig. 19, 20. Zwei kernhaltige Individuen im Zustand des Gleichgewichts.
Fig. 21. Ein kernfreies Individuum im Reizzustand mit deutlichen Anzeichen des Absterbens.
Fig. 22. Dasselbe kernfreie Individuum nach der Überwindung des Reizes.

Tafel V.

Fig. 1, 2. Kernfreier Teil, welcher Nahrung aufgenommen hat.
Fig. 3. Kernhaltiger Teil samt Nahrung.
Fig. 4. Kernfreier Teil.
Fig. 5. Derselbe kernfreie Teil mit aufgenommener Nahrung.
Fig. 6. Kernfreier Teil mit aufgenommener und einer andern gerade aufzunehmenden Nahrung.
Fig. 7. Derselbe Teil mit der gerade aufgenommenen Nahrung.
Fig. 8. Kernhaltiger Teil im Zustand des Gleichgewichts.
Fig. 9, 10. Ein kernfreier Teil im Zustand der Reizung und des Gleichgewichts.
Fig. 11. Kernloser Teil mit aufgenommener Nahrung.
Fig. 12, 13. Kernloser Teil mit den Symptomen des Absterbens.
Fig. 14. *Amoeba proteus* in vivo mit Neutralrot gefärbt, am Hinterende des Leibes den plasmatischen Körper samt Körnchen zeigend (*z*).



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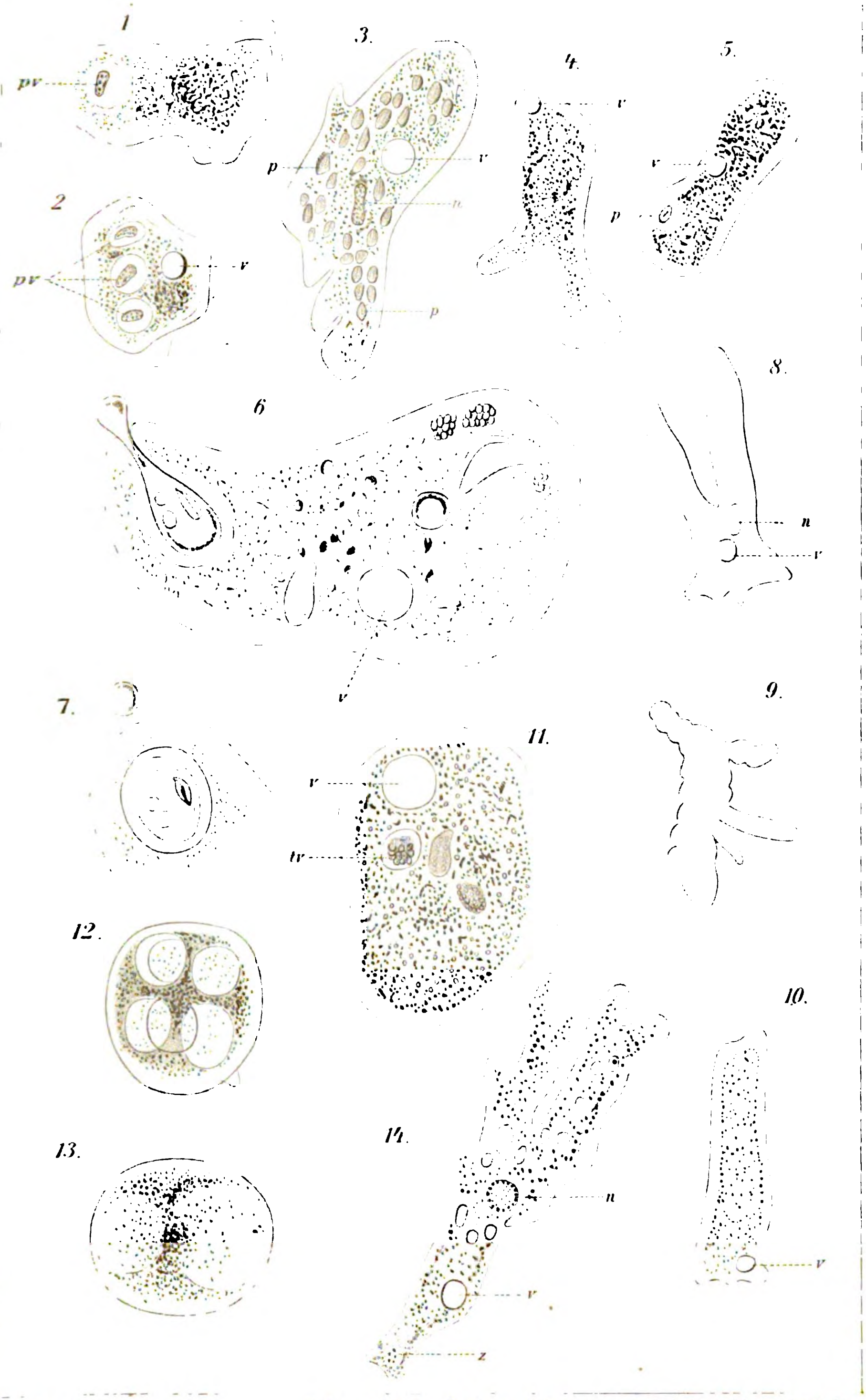

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8

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Ad finem provinciae Eiderstadensis septentrionalem, ubi regio inchoat editior, quae icitur vernacula Geest, haud procul a marchiis ad septentriones vergentibus, in ora atriae nostrae occidentem spectanti, nato mihi fieri non potuit, quin nomen febris archiae, inde a prima infantia memoria servatum, ante oculos saepe tulerit morbi amquam imaginem longe diffusi et metuendi. Cujus morbi casus quamquam in patriae niversitatis Kiliensis institutis clinicis rari tantum curantur, quum tamen occasio febris llius memoriam revocandi non deesset, consilium in animo est adamatum, tractando anc rem summum doctoris honorem adipiscendi.

Nova afferre nolui, sed dare volui febris descriptionem quam maxime proba-ilem et quatenus fieri potuit expositionem statisticam, quomodo in marchiis patriis aximeque Eiderstadiensibus illud genus febriculosum apparuerit. Quod conanti mihi ifficultates devincendas ne dicas parvas, positae sunt primum in hoc, quod casuum bservandorum nulla mihi ipsi oblata est umquam occasio inque primis, quod num-uam vidi epidemiam proprie sic dictam; deinde, quod quidem minoris est momenti, t hoc, quod satis magnus eorum numerus deest, quae sectione inventa sunt, quoniam, t postea videbimus, perpauci homines hac ipsa febri affecti mortem obeunt.

Itaque ea, quae sequuntur, e medicorum in Marchia optimorum relatis quum auserim, literas ad hanc rem pertinentes diligenti judicio magna cura subjeci, adjeci tem pauca tantum a me ipso observata.

Tractandi hic erit progressus, ut enumeratis literis primariis, primum morbum escribam, deinde aetiologiam, qualis observanda mihi videtur, instituam, denique ut, umbrata morbi quasi imagine, in uno ex omnibus, quae nunc probantur, nosologiae stemate ei locum suum tribuere operam dem.

Literae ad febrem, quae dicitur Marchiae pertinentes in Slesvigia Holsatiaque ucatibus observatam, sicut ad epidemiam febriculosam aestate anni 1826 grassatam:

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Christopherus Tinctorius, De febre continua Ao. 1640.

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Dr. Wendt, Kritisk Resultat angaaende en i Aaret 1826 *herskende Epidemie. K havn* 1831.

Mansa, Ueber die Kaltfie'erepidemie auf Lange'and 1826.

De febre Marschiae, quae dicitur.

(Nomina eidem morbo apposita lingua vernacula sunt: *Aehrenseuche, Stoppelseuche.*)

In febris genere, quod titulus perhibet, stadium prodromorum si apparet commodis gastricis diu manentibus atque levibus constat, nec non universa lassitu ut hominem pigeat omnis operae antecedentis. Usitatum est, quod febris pri apparet

I. ex stadio remittentis.

Comprehenditur homo subito, tempore, ut solet, meridiano, frigore jac succedente ardore atque sudore. Accedunt dolores vehementes et contrahent regione epigastrica, ructus, abdominis tympanitis, flatulentia. In plerisqne ca simul aeger de pressione ipsisque doloribus magnis in regione biliosa queritur giones et epigastrica et biliosa pressione irritantur et abdomen eflatum quider nec tamen dolorosum. Febris naturam induit remittentem; pulsus plenus et n ratus est et raris casibus apprime frequens. Deinde celerrime accedunt cetera o gastritidis indicia acutae. Crescit circa praecordia angor, sequitur nausea et post vomitus. Lingua crasse et late contegitur, sitis adest plerumque vehemens, tactu est calidissima, facies et oculi sanguine abundantes turgescunt. Primum cibo quod reliquum est vomitu ejicitur, deinde ejectum magis magisque fit subviride, sum, amarum.

Morbus rarioribus casibus in hâc gastritidis acutae similitudine sola acqui sed compluribus alia accedunt indicia, quae morbum ad gastroenteritidis similitud magis commutant. Sequuntur enim alvi dejectiones vehementes atque turbidae, i dum etiam quasi prosilientes, quales usitatae sunt in proctitide. Quae faecium eject onjunctae plerumque sunt cum tenesmo, nec raro observantur abdomen tumidum et latulentia, colica fere absente. Gastroenteritidis haec signa quum adsint, aegrotus de apite compresso et cephalgia vehementi queritur. Procedente morbo turbulentissime, ensuum errores, hallucinationes, deliria eaque furibunda saepe observantur. Corporis emperatura universa valde aucta atque elatior; lingua in siccitatem prona, sitis vehe-nens. Urina excreta pauca aut normali est specie, aut mediocriter excitata, mixta on raro cum acidi urinosi sedimento. Sin indicia tempore manent longiore, aeger aepe fit soporus, vel etiam comatosus. Cutis fit frigidior et sudore glutinoso conte-itur. Ipse pulsus minutior minus sibi constat, nec volente aegro faeces urinaque jiciuntur. Si sui adhuc compos erit aegrotus, de surarum tetano interdum queritur. — egari non potest magnam huic conditioni similitudinem inesse cum cholera nostrati. ulta tamen priori absunt indicia, posteriori quae propria videntur atque singularia.

Rei imperiti ipsique medici parum experientes non raro timent, morbo tam umultuose procedente, ne mors intret celerrima, quum medicus quisque magno usu raeditus, non nesciat, curandi quidem experimenta in hoc morbi stadio parum esse tilia, mortem tamen rarissime intrare. Medicus ad auxilium accitus aegrotum plerum-ue invenit hac gastroenteritidis acutae ratione affectum. Horas nonnullas post omnium ndiciorum remissio quaedam saepe sequitur, die autem sequente tempore, ut solet, re eodem, quam quo morbus primum institit, altera omnium signorum exacerbatio bservatur. Multis casibus ab initio ratio tertiana manifesta insita est, ut diebus im-aribus omnia indicia multo graviora appareant. Quod morbi stadium tres plerumque ebdomades, at interdum aut longius aut brevius susistit. Tempore procedente omnia ndicia imminuuntur, ut primis saepe diebus videantur vehementissima. Si conve-ientem aegrotus se habuerit, in hujus stadii fine omnia indicia acuta amissa erunt. ebris tum est minuta obtecta, appetitus deest fere omnis crebrarumque dejectionum ntestinalium loco obstructio apparet. Aeger se fessum et demissum sentit, sui tamen mnino compos conscientiaque est nullo modo perturbata. Cutis calore est pallido, epe lurido vel cadaveroso. Inde ab hoc tempore magis magisque manifestior ex-licatur febris natura et instituitur stadium consequens. Jam nunc lienis crescens emper magnitudo animadverti potest. Contra si neglectum se habuerit aegrotus, illud adium in longum tempus producitur, nulla febris forma manifesto apparente.

De hujus stadii complicationibus.

Signa cerebri, quibuscum stadium saepissime complicatur, ab initio saepe ap-rent, quum nos cohibeant, quominus veram morbi quasi imaginem intelligamus. aque non solum ansam dant, ut febrem cum typho confundamus, sed difficile etiam ddant, imprimis apud infantes a morbis cerebralibus febrem recte distinguere. ujusmodi complicatio tam est frequens, ut in superiore cum ceteris morbi indiciis a e descripta sit. Cujus causam, si inquiries ratione pathologico-anatomica, raro positam esse in meningitide et exsudatione inde proficiscenti, sed plerisque in casibus in nimia cerebri hyperaemia inesse inveniens. In qua tamen, maxime si arteriarum morbus simul adest, mors subita eaque apoplectica ingruere facile potest.

Pneumoniae etiam cum stadio febris supra descripto complicatio frebris est, ibi praecipue, ubi tubercula adsunt, quibus ad ineundam complicationem inclinatio quaedam primum insita est.

Inflammationem membranarum serosarum, pleurae, pericardii, peritonaei, quamvis ceteroquin rariores appareant, passim observantur perinde atque in endocarditide, si conveniet ratio quaedam epidemica idonea. Conclusio igitur ex analogia facta est, qua indicia cerebralia rara tantum ex membranarum serosarum inflammatione oriri in superiore professus sum.

Cum *rheumatismo* febris complicata rarius etiam observatur, et rarissime cum rheumatismo articulari; utriusque tamen rheumatismi forma interdum reperitur. Ceterum omnes fere morbi complicationes ineunt. Crebriores autem erysipelas et parotitis inire videntur.

II. De intermittentis stadio.

Stadio intrante aegroto adsunt omnia indicia, quibus intermittens in longum tempus producta apparere alias solet, concoquendi facultas imbecillima, appetitus absens, taedium et imbecillitatis sensus ceteraque alia. Cutis colore saepe est lucido, cadaveroso, livido, sicca, squammulis secedentibus. Abdomen saepe afflatum est, lien magnitudine crescere animadvertitur vel saepe tumorem exhibet magnam. Hepar etiam majus invenitur. Primo tempore febris forma intermittentis manifesta distingui non potest, quum crebro forma mutetur, post autem praecedentibus tribus vel quatuor paroxysmis minus claris, si fauste eveniet, forma constituta erit. Sin male se gesserit aeger aut mala erit constitutione, certa febris forma interdum nulla omnino apparebit.

Forma febricula creberrime observatur tertiana, modo pura, modo duplicata, modo duplex. Hannaei haec sunt verba: „quantum hactenus observavi, febres referri possunt, vel ad tertianas, modo continuas, modo duplices, modo duplicatas, modo anomalas.“ Rarius observatur intermittens quartana, numquam fere quotidiana. Forma febricula anteponens sive postponens creberrima est. Itaque in hoc stadio saepissime invenitur febris intermittens tertiana anteponens sive postponens. Si febris forma manifesta exculta erit, temporis momentum ad adhibendi curandi rationem idoneum aderit et plerumque tum continget, ut remediis febrifugis lege artis adhibitis ipse morbus depellatur. Sin autem aegrotus male se geret sive tenui erit constitutione, certa febris forma multis casibus aberit, iisque quidem difficilibus, qui curari et prognosi distingui possint. Tum enim omnia apparent indicia, quae morbos sanguineos interque hos imprimis intermittentem consequi novimus. Quae in posteriore afferentur, ubi de morbis febrem consequentibus sermo erit. Jam vero morbo curatione prosperrima depulso, interdum tamen mali morbi consequuntur; certum saltem st, infirmitatis sensum animumque depressum longiore tempore restare, partes, per as concoquitur, diu esse hebetatas hominemque in universum ad aegrotandum esse ronum. Qui quidem facillime febri recidiva afficitur, de qua postea sermo erit, ubi e ceteris morbis consequentibus dicemus.

De morbis febrem consequentibus.

Febris recidiva quum morbus iteratus tantum sit, non oportet amplius tractetur. lemorandum tamen est, in recidiva stadium remittentis brevius plerumque esse nec eque; ac in primo morbo, tumultuosum procedere, recidivam autem propter constitionem praecedenti jam morbo turbatam, quum et processus minime sibi constet, ifficiliorem semper esse, quae sublevetur. Deinde *tumores lienis* semper fere febrem nsequuntur, unde fit, ut et in nutriendum et maxime in sanguinem comparandam embrum morbosum mutuam vim suam referat. Quod quae sequuntur, apparent in ccis viribusque corruptis, saepe in morbo hydropico, nec raro mors, praecedentibus jusmodi signis, intrat. — *Morbus Brighti* saepe hydropi suppositus esse videtur, per tamen febrem raro consequitur. — *Hepatis affectiones morbosae*, febrem non raro nsequentes, ex magnitudine hepatis, modo magis modo minus aucta apparere con- st. Telarum mutatio constare censetur tum in eo, quod vernacula dicimus lingua *peckige Entartung,“* tum in mutatione quadam ad id, quod dicimus *„fetthaltige Muskat- ssleber.“* Quas degenerandi formas vel semper consequetur universa cachexia, cre- rrime autem hydrops, cujus origo posita plerumque erit in ascite. — *Tuberculosis.* ximo periculo febris genus, de quo dicimus, iis hominibus esse solet, qui ad phthisin turam tamquam hereditate pronam habent, aut qui ea jam affecti sunt. Tuberculosis febri orta crescit plerumque vehementissima, quod quidem ex febris vi in nutrien- m male valenti explicari debere verisimillimum videtur. Sed phthisis interdum in- stat homines adhuc sanos, nulla causa tamquam hereditaria ad eam inclinantes. — teri morbi, qui consequuntur, plurimi ex singula quaque praecedentis febris compli- tione profiscuntur, exempli gratia cordis vitia. Qui quum parum sibi constent, inter os morbos consecutos numerari vix possunt.

Quae instituta sectione observata sint.

Quum aegros febri Marchiae, quae dicitur, affectos raros tantum, eosque ne a quidem febri, sed aliis morbis eacum complicatis, vel fortuito accedentibus ten- os, mortem obire in superiore dixerim: sectionum institutarum numerum parvum, ne quae institutis illis in lucem prolata sint, pauca tantum esse necesse est. eram equidem dedi, res quam plurimas e diversis epidemiis a medicis compluribus, tituta sectione, disquisitione pathologico-anatomica collectas inter se comparare. ae, quamvis numero paucae, probant tamen, id quod sectione maximum repertum

sit, cum illo omnino convenire, · quod intermittentem malignam sequi soleat,
ratione etiam anatomica febrem marchiae eandem esse atque febrem intermitt
constet. In quo quamquam confiteri debeo, me propriis oculis nullam post f
illam mortui sectionem inspexisse, contendo tamen, talia in ea numquam obse
qualia febrem cum typho eundem esse morbum testentur. Itaque quae de sectio
anno 1847 institutis tradidit Löfflerus, chirurgus militum, quum fere in omnibu
mores intestinales · typhosi apparuerint, ita impugnare velim, ut eos, qui secti sint,
febri illa, sed typho mortuos esse contendam. Quod eo magis velim, quum m
Eiderstadensibus aestate ejusdem typhi certe casus, nec tamen febris illius cur
fuisse audiam.

Ea, quae sectione apud eos in stadio remittentis, qui mortui erant, inventa
omissa complicatione, quam cujusque fere mortis veram causam fuisse demon
potest, haec fere erant: in *cavo cerebri* meningae et sinus, cerebrum ejusque
sanguine abundantia, ventriculi cerebri plerumque liquore seroso pleni, in *cavo*
minis lien et hepar magnitudine magis minus normali, sed mollia et sanguine co
ductus choledochi saepe obturati. Stomachus cum cetero tractu intestinali di
inter se ratione inflammati inveniebantur. Glandulae tractus intestinalis in si
quidem casibus tumidae erant; nec tamen ulcera intestinalia locum obtinuisse tra
est. Renes tum hyperaemiam, tum primum stadium morbi Brighti exhibebant,
erant — idque saepissime — conditione normali. In *cavo thoracis* organa pleru
erant sana, aut, prout complicatio singula semper aderat, affecta.

Quae sectione apud eos inventa sunt, qui in stadio intermittentis mortui
adeo cum iis, quae intermittenti vulgo reperiuntur, convenerunt, ut sat esset
ipsa memorare.

Prognosis.

Prognosis in singulis casibus diversissima est. Fausta institui potest, si ho
alioquin sani aetate sunt media, contra gradatim infaustior erit, prout condit
erunt minus convenientes. Morbum semper pendere a nutriendi turbatione vehe
in longum tempus producta, in superiore verbo jam attigi. — Recidivae igitur
gnosis propterea erit minus prospera, quod nutriendi turbationibus corpus di
propter morbum praecedentem, expositum fuerit, in quo causa etiam est, cur et m
difficilior sit, qui sanetur, et crebriores morbi consequantur. Casus in tempus
rogati eorumque hominum morbi, qui infirma tantum sunt constitutione, duplici r
infausti sunt. Altera quum non raro accedat, ut nulla febris forma manifesta app
itaque temporis momentum certe observare vix possis, quo sanandi ratione o
inchoes, quo fit ut morbi sublevandi finem difficile assequaris; altera quum ad e
recidivas tum inclinatio saepe sit magna morborumque numerus consecutorum
parvus. Complicationum perinde atque morborum consequentium prognosis pro g
diverso diversa erit. Itaque in universum febri marchiae in rerum conditio

stis prognosin etiam prosperam institui posse contendo, quum; si contrariae res nt, praevideri non possit, quae futura sit vis ejus damnosa.

Therapia.

Prophylaxis eo constat, quod epidemiis grassantibus homines nondum affectos archia amovendos curatur. Quod quidem semper fieri non potest; ubi tamen poterit, minibus imbecillis ad tuberculosin inclinantibus, id maxime suadendum est.

Curatio, in utroque febris stadio diversa, a medicis marchiam habitantibus tione fere semper eadem adhibetur.

In stadio remittentis — idque maxime in casibus non praeter usum proceden-bus — gerendi ratio ad regulam quandam exaequanda tantum est. Cures enim grotos in cubiculo bene temperato, sicco, aëri pervio cubandos, lecto tranquille nendos. Cibos proponas leves, ad solvendam alvum non idoneos, jura imprimis maria parva multitudine. Medicina non opus est, praebere tamen potest solutiones tri bicarbonici, saturationes, pulveres aerophoros, aquam Selters. Alia sicut emetica, rgantia, apta tantum afferuntur in stadii prodromo, rarius observato; morbus post-am ingruit, male illa feruntur. — Alvum obstructam auferet ol. ric. cochlear. omitus si minus est, a plurimis medicis proponuntur potiones, quae vernacula di-ntur „*kohlensäurehaltige*,“ aut tincturae antispasmodicae, quarum sunt: tinctura Valer. th., liq. c. c. succ., liq. Ammon. anisati et cetera. Alii glaciem adhibitam, sive lulas, quae dicuntur „*Eispillen*,“ magnae utilitati esse profitentur. Alii hyperemesin blevare conantur remediis derivatoriis, regioni abdominali applicatis, cucurbitis. arrhoeae nimiae difficiliores etiam sunt, quae curentur. Opium abhibendum non se omnes fere medici inter se conveniunt, quippe quod cerebri congestiones, jam ae adsint, augeat.

Plerique medici liquores acriores applicando, aut sinapismos abdomini infri-ndo prodesse student. Adhibentur etiam clysteres amyli et opii. Diarrhoeas im-gnare medicamentis internis jam propterea difficile est, quoniam simul adesse solet mitus vehemens, quo medicina statim ejicitur. Febri dominante vehementissima nulque si magna cerebri symptomatum ratio aderit, utile erit, cerebri hyperaemiam impugnare, quod cataplasmata frigida, sacculi glacie impleti adhibentur hirudines-e regione temperali applicantur. Exitus lethalis si complicatione aliqua impendet, mediis singulo cuique casui convenientibus agendum erit. Cunctis casibus unum medium, sive castoreum, sive mochum, sufficere cum medicis nonnullis marchiam bitantibus vix consentiam.

Stadium intermittentes. Ipsa curatio medica in hoc febris genere tum demum choat, si febris forma manifesta exstare coepit, vel ut rectius dicam, manifesta con-tuta erit. Quum indicia tumultuosa et gastrica fere omnia amissa sint, perturba-nes tamen tractus intestinalis saepe adhuc adsint, remediis, remediis febrifugis

praemissis, concoquendi facultatem meliorem reddere medici solent. Quem ad
praebetur natrum bicarbonicum, chlorammonium, remedia amara ceteraque pauca
tamen licet ipsum febrifugum nimis cunctari, quod quidem semper fere bene f
Febrifugum plerumque est chininum; alia febrifuga quae secuta sint nescio. In
bendo chinino rationem medici sequunter in intermittenti usitatam, at utile est,
fugum ratione prophylactione diebus usitatis reiterare. Quomodo semper fe
morbum tollendum satis valet. Quo sublato, curatio subsequens accurate ad
debet, et quae ad cibos, sanescenti praebendos, et quae ad totam gerendi rat
pertinet, quum alioquin non solum tardior sit convalescendi progressus, sed
recidivae aliique morbi instent. Difficile esse potest, adhibendi febrifugi mom
recte judicare, si vehementer febris aut anteponit aut postponit. Febri magis
dente, quum certa plerumque forma appareat, utilius esse dicitur, febrifugi us
aliud tempus differre. Casibus nimis productis aegrisque malae constitutionis chi
adhibitum non sufficit. Saepe chinino cum opio aut cum remediis amaris con
id saepe assequeris, quod solo chinino non potueris assequi. In aliis etiam ca
imprimis postquam chininum ratione convenienti jam adhibitum est, febris eo vi
quod chinini usus in tempus differtur satis posterius, aut quod, si antea por
datae sunt majores, tum portiones praebentur minores et sic ex contrario. Ul
refugium, perinde atque in febri intermittenti tenaci in eo inest, quod aegro
aliam terrae regionem, eamque quidem editiorem, quae *Geest* dicitur, a marchia
maxime remotam, transfertur.

Recidiva simili ratione, qua morbus praecedens, curanda est. Morborum
tio consecutorum alia non est, quam quae universa lege therapeutica ad eos pert
praescripta est.

Brevia quaedam de epidemia longius atque vehementius grassata ne de ratione, quae inter febrem Marchiae, quae dicitur, intercedat et f in regione aequinoctiali grassatas.

Descriptione mea superiore genus febriculosum post Marchiam ditum ita
quale et in plerisque epidemiis minus gravibus et maxime in provincia Eiderst
inde ab anno 1826 observatum sit.

At animo conspiciemus morbi quasi imaginem multa ratione aliam, si
dierimus, quae de epidemiis gravissimis, quae grassatae sunt anno 1614, dein
1640, mox an. 1699, tum an. 1734, postea an. 1791, denique an 1826 obser
literis tradita sunt.

At epidemiae indiciis et processu inter se ita differunt, ut de singula ep
in omnem partem satis hoc loco non possit tractari. Epidemia anno grassata
ea quidem optime descripta, haec fere fuisse nobis videtur. Exstitit per te

spatium inde ab ultima mense Junia usque ab mediam mensem Decembrem. Morbi processus, quam maxime diversus, saepissime hic erat: Aegri vehementi et animi et corporis depressione affiiebantur. Qua postquam diutius aut brevius occupati sunt, in ipsum morbum inciderunt, ingruente vehementi febri remittente, illi non dissimili, quam in superiore descripsimus. Signa gastrica plerumque non erant tam gravia, et insignis febris remissio vulgo observabatur. Exacerbationes non semper vesperi, sed aliis quopue diei temporibus apparebant, conjunctis fere semper vomitu, angore, frigore, qui quidem cum ardore mutabatur, nec raro erat vehementissimus. Praeterea morbo procedenti ratio, quae dicitur, tertiana aderat, ut febris incidiaque consecuta diebus imparibus graviora essent, quam paribus. Dies plerumque quatuordecim postquam processit, morbus transire solebat in stadium quoddam, ut verbo dicam, typhoide, cujus post exitum quum apparuissent omnia typhia signa, aut valetudo denique restituta est, aut mors intravit. Nec tamen morbum fuisse aut ipsum typhum, aut stadium typhoide tale, quale in cholera morbo observari potest, satis testantur de corporum sectione inde ex illo tempore literis tradita, maxime memoranda illa Frickii de epidemia Groningiana, illaque Hachmannii de epidemia Ritzebüttelam infestante, praefecturam Hamburgensem.

Quamquam haec quoque epidemia tam diversum morbi processum singulis casibus exhibuit, ut indicia fere omnia, quae in febribus apparent intermittentibus, observarentur.

Tanta indiciorum varietas, regnante epidemia graviore, memoriam nobis affert illorum, quae de febribus in regione aequinoctiali grassatis Senac (l. c. p. 158 sqq.) descripsit, qui quidem ex una epidemia variisissima perhibet signa observata, utpote, quae alias colligas necesse est ex omnibus, quae de febribus, aestuosas terrae regiones infestantibus, passim observata sunt. In universum genus febriculosum in Marchia, qualis est ejus natura in epidemiis vehementissimis, febri simillimum est in regione grassatae aequinoctiali.

Quum longum esset, singulam quamque epidemiam febriculosnm, quae Marchiam infestaverit, hoc loco describere, in superiore eae tantum literae allatae sunt ad rem pertinentes, quas propriis oculis inspexi. Librorum illorum, quibus febres in terris grassatae peregrinis, maxime in regionibus, a circulo aequinoctiali proximis, describuntur, hi in manum mihi venerunt:

Fricke, Bericht über seine Reise nach Holland 1826.
Fricke, zweiter Bericht über die Epidemie in Gröningen 1827.
Ludwig Hermann, über die Wechselfieber in Algier. Frankfurt a. M. 1836.
A. Haspel, maladies de l'Algérie 1836. 51.
F. C. Maillot, recherches sur fièvres interm. du Nord de l'Afrique 1835.
Rob. Jackson, Fieber in Jamaica 1796.
John Hunter, Truppenkrankheit in Jamaica 1792.

W. Arnold, a pract. treat. on the Bilious Remittent Fever. London 1840.

John Clark, Beobachtungen über die Krankheiten in den heissen Gegenden. London 1792.

Comparatis iis, quae de febribus scripta sunt in regione aequinoctiali grassatis, cum iis, quae de nostris febribus intermittentibus relata sunt, si exploravimus ea, quae inde sequuntur, maxima videbitur harum febrium signorum inter se similitudo. Neque enim solum conditiones soli ceteraque momenta aetiologica inter se simillima sunt, — nam loci palustres et aestates usitatis calidiores, praecedentibus inundationibus ad utrum febrium genus producendum plurimi valent — sed etiam in universo signorum apparentium ordine mirum quae sit aequabilitas. Quamquam hepar illis febribus in regione aequinoctiali maxime affici vulgo putatur, dum apud nos membra respirabilia febri affecta magis inveniuntur: hoc tamen discrimen inter illas febres et has, quae marchiam infestant, earumque epidemias vehemensissimas fere nullum est. Nam natura biliosa febris in marchia grassantis quum in universum animadvertitur, tum magis etiam in epidemiis vehementissimis praevalet. Quamvis hepatitis acuta, hepatisque abscessus in hoc genere febriculoso non observati sunt, continuus tamen in regione hypogastrica dextra color, icterus non rarus, tumor hepatis, qui observabatur saepe post morbum sequens, sicut aliae circulationis hepaticae turbae manifestissime ostendunt, hepar quoque hoc genere febriculoso vehementer affici. Alteram etiam similitudinem quandam cum illis febribus in regione aequinoctiali febris, quae marchiam incadit, communem habet. Nam ratio, qua magnae epidemiae terram invadunt, tam diversa atque varia esse solet, ut anno 1826 multi auctores, inter quos Friedliebius Husumensis, Dohrnius Heidensis, judicio vacillaverint, essetne febris, an typhus, an alius morbus adhuc qui nondum adfuerit, an potius genus morbosum utroque illorum mixtum. At virorum doctorum Louis, Hübbe, Reye de epidemia aestiva anni 1826 haec est sententia callida: febrin Marchiam invadentem, si nomine comprehendi debet genus perhibenti, nec esse febrin biliosam, nec febrin mucosam, nec typhum, quum morbi indicia, quae his nominibus amplecti soleamus, apud omnes aegros in universum nequaquam apparuerint, sed a singuli cujusque aegroti natura et vi magis pependerint, quam in ipso morbo posita visa sint.

Iidem viri, quum epidemiam in Germania septentrionali grassatam comparare conati sint cum illa in Hollandia grassata, probatum instituerunt, utramque uno genere comprehendi, temperatam quidem singulam esse effectibus endemicis, omnemque epidemiam momentis epidemicis auctam atque graviorem factam, ut verbo dicatur, intermittentem.

Generis febriculosi Marchiae, quod dicitur, aetiologia et genesis.

Probatum est, morbum singulis casibus sparsisque numquam apparere, quocum omnes, quos ipse adii, medici convenerunt. Quaerenti mihi medicus in Eiderstadia celeberrimus: pro certo habeo — inquit — si hodie primus morbi casus mihi curandus erit, curandi copiam proximis diebus post utraque manu me occupatissimum habituram esse. Morbus vehementer ingruere vulgo coepit epidemicus annis 1614, 1640, 1699, 1734, 1791, inque primis an. 1826; evenit mitior ceteris annis, quum vel multorum annorum intervallis Marchiam omnino non infestaverit; sic nunc deest Eiderstadiae morbus inde ab anno 1847. Ex eo intelligas, quanta vi morbus provehat, quod Friedlieb physicus anni 1826 mense Augusta nullam in Eiderstadia domum fere fuisse dixit, quin homines febri affectos in se haberet, immo domum felicem praedicari debuisse eam, qui vel unum haberet hominem febri liberum. Hominum 5367 Ritzebütteliam praefecturam habitantium anni 1826 aestate autumnoque febri afficiebantur tribus millibus ducentis triginta sex non pauciores.

Causas explorare, ex quibus epidemiae omnis impetus semper pependerit, imprimis certe judicare, quatenus terrarum conditiones ad epidemiam pertineant, perdifficile est. Si alteram in partem negare nequimus, solum Marchiae lutosum ad proferendam febrem multum conferre, quum regionibus mari quidem adjectis, sed editioribus, morbus numquam appareat, in alteram partem in hoc posita omnis causa esse non potest, quoniam complurium annorum intervallis genus illud febriculosum abfuisse videmus.

Causas quum investigarim, quae essent ex quibus maxime febris penderet, quamvis dubium illud miasma, quod dicitur, invitus in memoriam redegerim, non potui tamen, quin in hoc omnem causam posuerim. Etsi enim de miasmate sententias illas tamquam pueriles, quarum auctores ante hos triginta annos vixerunt, Friedlieb aliique medici aequales, non sine risu nos despicere solemus: despiciendo tamen nulla explicandi ratio alia oblata est, quare quum miasma, idque palustre vel lutosum, hoc loco sine ulla dubitatione instituam morbi causam, id intelligo tale, a quali proximus pendeat morbus, — verbum tantum pro re, quae accuratius definiri nequit. Itaque quum causas afferam, quae miasma producere possunt, eas simul intelligo epidemiam quoque producentes.

Harum causarum genus, quod dicitur praedisponentium a genere, occasione certisque conditionibus ad epidemiam invitandam allato distingui potest. Illud genus causarum praedisponentium, vel ut verbo utar graeco, endemiarum, intelligo ex terrae situ et soli conditione, sicut ex habitantium moribus oriri. De quo breviter afferam haec:

A. De causis praedisponentibus.

Accurata locorum in Marchia sitorum descriptio non pertinet ad rem, hoc tamen huic loco apte inserendum videtur.

Post ingentem eruptionem, qua ex interioribus terrae continentis partibus in mare aquae ante haec millia annorum prorupisse putantur, quum ad finem haec terrarum turba venisset, naturali factum est necessitate, instante iterum tranquillitate, ut partes non solum ex terra continenti orae patriae nostrae, in occidentem vergentis, abruptae, sed etiam fluminibus secum ductae iterum quiescerent. In his propter pondus saxa partim ad maris, partim ad fluminum fundum primum abscesserunt. Quibus tardius sabulo resedens, fluctibus marinis ad ripas, integras relictas, depositum, fundum formavit marchiae. Terrae partes subtiliores et leviores, argilla, latum, calcis partes non paucae, sicut eae etiam partes, quae aqua poterant solvi, priusquam resederunt, diu mevebantur, neque, nisi rerum ordine prius restituto maris quotidiano accessu et recessu ab aequis poterant secerni. Nec aliud contigit partibus vegetabilibus, aquae propter levitatem innatantibus. Quae simul cum partibus terrosis a fluctibus in sinus dejectae, et terrae continenti et fluminibus alte injectos, aestu maris decrescente magnis molibus cum terra mixtis, relictae sunt, unde terra orta est nostro sermone, quae dicitur „*Moorgrund.*“ Super has straturas ex partibus terrosis et vegetabilibus constantes, fluctuum processu innumerabilium et crescentium et decrescentium nova purae terrae stratura assedit. Et haec ipsa Marchiae origo est, cujus inter inferiorem straturam subulosam et superiorem lutosam suppositum est ipsum solum, quod dicitur „*Moor.*“

Marchia post illud tempus eodem modo orta est, quo oritur etiam nunc, quod ex terrae continentis partibus interioribus in mare continuo a fluminibus ductae subtiles argillae, soli, sabulonis, calcis partes, ad terram vel super terram, adhuc jam constantem, sternebantur. Terra, super quam marchia nostro tempore prosternitur, verbo dicitur vernaculo „*Wattland,*“ aggeribus nondum circumclusa, mari adsita, cujus aestu quotidiano inundatur. Cui terrae solum etiam suppositum est, quod dicitur „*Moor,*“ ita, ut locis quibusdam, qui donec aestus crescit, inundati sunt, decrescente aestu, turfa, quae dicitur, fodi possit.

Marchiae nostro tempore formatae ab illa distinguendae aggeribus multo tempore ante circulosa, nomen datum est vernaculum „*Seemarsch.*“ Omne tamen discrimen, ut ex iis apparet, quae supra dixi, positum est in eo, quod inde a locorum finibus editiorum, qui „*Geest*“ nominantur, usque ad mare stratura lutosa crassitudine sensim augeatur, quo modo fit, ut prope a finibus illis paucas jam pedes sub summa terra solum inveniatur illud, quod nostro dicitur sermone „*Moorboden,*“ quum ad oram marinam straturae reperiantur 16 vel 20 pedes crassae. Itaque manifestum est, in omni ducatuum nostrorum marchia tres esse straturas crassitudine sola inter se diversas. Deinde apparet ex iis, quae supra de marchiae origine exposui, mirum non esse posse, si locis quibusdam majoribus vel minoribus unam e tribus straturis, quas dixi, aberit; — abesse enim non raro videtur stratura illa, quae vernaculo dicitur „*Moorschicht.*“

Praemissa descriptione, brevi illa de terrae natura et situ, mirum non videbitur, febris genus, illi palustri simile quoddam, ex marchia originem duxisse. Quid enim? reperire in marchia solemus sub stratura lutosa, subluto, argilla, marga, solum quoddam uliginosum vel palustre. Unde insignis vegetabilium copia ad putescendum aptorum. Dum straturis lutosis, crassitudine diversis, magnae straturae palustres passim substratae sunt, solum et supra has et supter omnino ita est constitutum, ut aquas stagnatas facile in se inclusas teneat. Ex straturis palustribus et ex aqua insignis vegetabilium putriscentium copia oriatur necesse est. Inde proficiscenti respirationi evadendi occasio affertur partim stagnis quibusdam; in locis praesertim demissoribus non raris, quorum nomina illic habitantes sua perhibent lingua „*Wehlen*, *Tiefen*, *Spätingen*,“ partim fossis per omnem marchiam frequentissimis. Ne praetermittam, quod ad similitudinem locorum palustrium timuendorum in terra, gramine vacua, sicut in itineribus hiatus quosdam altos et latos saepe vides, solis ardore factos, ex quibus respiratio maligna facile potest exire.

Memorandum hoc loco etiam est, quam sint hominum domicilia in Eiderstadia locis omni ratione incommodissimis plerumque condita. Quamquam solum maxime est humidum, fundamentum habitationibus substruitur minimum, domus pleraeque unam habent tabulationem itaque conclavia, quae plano pede sunt, quam maxime humida, post ut ineptiis quasi cumulus afferatur, arbores circa domum plantantur ultimam soli humorum exurendi occasionem auferentes.

Notum esse aestimo, quam bibendam ex aqua in fossis stagnata hauriri, quam percolando non purgari constat. Aliud etiam, quod observatum est, ad rem pertinet. Ebriositas quaedam et voracitas, ex rerum familiarium copia quadam et ubertate alitac dominantur, et eo sunt perniciosiores, quo minus earum patientes sunt, ne dicam homines ipsam Marchiam habitantes, alii tamen non pauci, qui messium tempore e locis, qui „*Geest*“ dicuntur, in marchiam migrant; immo omnibus eo ipso perniciosissima sunt vitia, quod messium mensibus calidissimis maxima libidine evagentur. Jam circumspiciendum etiam est singulare aliquid, quod maxime in Eiderstadia obtinet. In usu enim est laborum per diei spatia distributio parum salubra. Ut operarii quam maximae utilitati sint, horologia ita ordinatur, ut false tempus binis circiter horis serius indicet, quam vero est, quo fit, ut illi aestatis noctibus frigidis nimis mane, exempli causa, quum hora post mediam noctem quarta deberent, hora jam altera laborem adeant, quum hora duodecima deberent, hora jam decima coenent itaque dies tempore calidissimo inde ab hora duodecima usque ad alteram post meridiem horam laborent.

Afferre omnes causas tam in terrae conditionibus quam in populi moribus et consuetudine positas, longe erat. A quo desistens a causis praedisponentibus jam ad singularum epidemiarum causas occasione certisque eventibus allatas exponendas me converto.

B. De causis occasione allatis.

Causas occasione allatas post id, quod supra dixi, intelligo oriundis singulis epidemiis, quae sunt rerum conditiones datae, ansam afferre. Quam ad rem quae pertinent duo observanda esse et omnes de hac re auctores profitentur, et quisque peritissimus affirmat, *duo momenta*, quae marchiae maximae esse vox etiam populi est. In alteram partem sunt aqua inundationes marina pra sive autumno sive vere, maxime ei delapsus, quo opus est, non habent, effusa stagnet. Sunt tamen etiam aquae dulces post imbres et nives cont magnos coeuntes, quae praecedente sive autumno sive vere a coelo delap In quo id, quod aquarum derivandarum ratio annis postremis tam exculta in liorem conditionem instituta est, praeterea, quod aetates eorundem annoru erant frigidiores, maximi ad amovendas epidemias febriculosas annis recentiss momenti. Inundatio sola tamen ad producendam epidemiam non sufficit, q potius febri oriundae tempestas vehementer humida obstet, nec post maxim dationes secutas esse epidemias saepe videmus. Accedat igitur necesse es quoddam momentum *alterum*, quod est temperatura vehementer aucta atque Itaque undique probatum est atque certissimum, aestatibus solis calidis invas demias, easque perniciosissimas ingruere, si forte post autumnum sive ver undatione abundans, aestas sequitur calidissima. Probari deinde potest, pri semper febris casus in regionibus apparere, quae erant inundatae. Epider 1826 vel pessime auditur, ubi post inundationem an. 1825 universe effusa secuta est calidissima. Afferre possem plura etiam testimonia. Quidnam sit, haec caloris vis pestifera praecedentibus inundationibus posita sit, id ipsum ostendere, difficile est, nec equidem plura afferre audeo, sed singula tantu magis indicantia, quam illustrantia. Naturale fit, ut vitia hominum incole superiore dicta, voracitas illa mosque nimis mane lecto surgendi, regnante c reddant perniciosiora. Accedit, ut aqua, vegetabilibus completa, majore calor ita putescat, ut saepe aquam bibendam aestatis tempore invenierim colore non raro male redolentem ipsamque foetidam, infusoriis plenissimam. Deinde tam, potiones etiam alias ab operariis in agrum secum portatas celerius atqu corrumpi acidasque fieri, inter quas cerevisiam.

Afferenti mihi haec omnia momenta ad epidemiam producendam vale maria tamen causa in alia re posita esse, a calore etiam probata, videtur. Q inter omnes illos, qui in marchia nati sunt, non vidit, vaporum straturas hu binos vel tres pedes altas, super summam terram stratas easque vesperibus multos pedes altas, albidas, ut, qui permeat, non solum pedibus, sed toto co uvidus. Vapores plerumque foetidissime redolent, quorum in partibus arte disquirendis hoc loco ne nimis mores, hoc solum contendo, praeter partes palustri vulgo insitas, nullas inesse ei proprias et singulares. Itaque quamvis hujus miasmatis naturam et indolem a me non esse exploratam, vim ta damnosam ex eo intelligere velim, quod et domus habitatienesque et vias via vapore illo humido tamquam velatos esse vidi. Quanam vero ratione ca vaporum multitudinem nimiam super terram colligere possit, ne id ipsum explicen facile quisque mihi condonabit.

Nomina manifesto indicant, quae populus sua lingua febri tribuit „*Kehrenseuche Stoppelfieber*,“ in frugibus ab agris amovendis aliquid inesse vulgo putari, quod epidemiae faveat. Quod non sine ullo jure putari ex eo apparet, quod frugum metendarum tempore epidemiae origo esse solet. Quod ipse mihi explicare velim hoc modo Dum enim pascua solis radiis faciliorem in se aditum permittunt itaque cohibetur quominus humores majores accumulenter, agri frumentarii solis radiis in inferiore partes introitum prohibent. Simulatque frumentum metitur, et copiosiores sunt humores solis calore vaporantes et vapores hominum valetudini eo perniciosiores, quo diutius humores in locis suis constiterunt. Itaque epidemia plerumque optima messium mensa Augusta ingruit.

Epicrisis.

Post ea, quae supra instituimus, si febrem marchiae, quae dicitur, certo cuidam morborum systemati inserere conabor, id mihi tum bene successurnm esse censeo, si et omnem ejus processum — eventum intelligo in intermittenti — ut rationem aetiologicam semper bene respexero. Quam quidem rationem aetiologicam morbi intelligo originem, ex locorum certis conditionibus ad malariam producendam idoneis. Quas conditiones marchiae non deesse in superiore satis me probasse puto.

Definiendo me liberatum quemque habiturum esse existimo, qui sint morbi quibus in universum hodie nomen tribuatur vernaculum „*Infectionskrankheiten?*“ deinde quae sit inter hos malaria? Nemo contendet, febrin marchiam invadentem non esse morbum miasmaticum, qui dicitur, sed contagiosum; — quoad tandem discrimen, quod sit inter morbus miasmaticos et contagiosos omnino certum institui vere potest. Morbum enim ab uno ad alterum transferri, materiamque contagiosam certam quandam in homine, qui aliunde ea afficiatur, crescere, in genere febriculoso, de quo sermo est, eadem ratione monstrari nequit, qua in typho et cholera.

Conabor febrem marchiae, quae dicitur, inter morbos, qui vernaculo *Infectionskrankheiten*, eam malariae speciem instituere, cui tempore recentiore nomen *febrium malariarum remittentium aut continuarum* viri docti apponere coeperunt.

Morbum malarium illam febrem habeo, primum, quod originem suam ducit ex eodem causarum genere singularium, non contagiosarum, sed miasmaticarum, ex quo febris intermittens proficiscitur, deinde quod cum hac illi singularia quaedam in turbationum ratione aequa sunt, quatenus haec disquisitione anatomico-physiologica adhuc explorata sunt, praecipue denique quod inter utramque insignis indiciorum similitudo est et utrique inest ad paroxismos febriculosos inclinatio, qui ordine quas rhythmice procedunt. Quod quidem incipiente febri illa manifestum non exstat, sed indicatur tantum, procedente autem illa certius apparet et verum signum est.

Iterum febris illa cum ceteris malariis hoc etiam convenit, quod incertum videtur, ubi sedem habeat et quod sedem habet diversam. Pertinet quidem imp ad hepar, ad lien et ad tractum intestinalem. Probare nequeo, cum magna ta certitudinis specie suspicor, in hac etiam febre corpuscula sanguinis alba rubro impedio augeri nec in gravioribus febris statibus inveniatur. Itaque si in iis, q sequuntur, febrem illam morbum dicam malarium, amplius demonstrare operam da *febre esse illam intermittentem complicatiorem, ipsis complicationibus quae difficile cog scatur.*

Reliquum est explicare, quid inter febrem illam et typhum intersit, res quid nullo modo difficilis. Eo praetermisso, quod ad oram patriae nostrae occident spectantis, imprimis in marchia, typhus morbus videtur rarus, ingruendi ratione mi mam habet altera cum altero similitudinem. Quis de typho cogitabit, praesertim typho ileoso, si omnes in marchia medicos audierit inter se convenientes, ne unu quidem febri illa aegrotum sibi mori. Quod si afferimus, nunc omittamus licet ratio nem supra redditam, quot fuerint mortui in magnis epidemiis, exempli gratia ann 1826. Sed ipsum illud, quod e medicorum omnium de febre audivimus, tale es quale e levissimis quidem epidemiis typhosis possumus tribuere. De typho aute exanthematico idcirco cogitari non potest, quod ipsum exanthema, in quo positum e magnum discrimen, febri illi omnino abest.

Ut amplius exponam, quid inter utrumque morbum intersit, hoc etiam afferro Typhum interdum genere febriculoso intermittenti institui id non nescio quidem nescio tamen typhum procedentem denique in febrem intermittentem plane mutatum exire posse, quod quidem in febriae Marchiae semper ordine fit. Deinde si nega nequit, quod observatum est, intermittentem, febrem marchiae, typhum simul eode tempore invasisse, ex hoc tamen typhum et febrem illam unum eundemque esse mo bum colligi nullo modo potest. Sin autem veteres nonnulli medici febrem illam i typhum transiisse dicunt, morbum aut typhum a prima origine, aut genus febri Marchiae complicatum fuisse contendo, cum typho nulla ratione conjunctum sed statum quendam typhoidem, qui dicitur, cui tamen indicia plane abessent, quibu typhus constitueretur. Sed hoc vel posito et concesso, fieri posse, ut cum typho febris illa complicetur aut in typhum exeat, tantum abest, ut hoc ipso utrumque morbum unum eundemqne esse probetur, ut id solum effici sentiam, binos morbos qui sub nomine vernaculo *„Infectionskrankheiten“* recentiore aetate viri docti un attribuant ordini, eodem tempore invadere et unum eundemque hominem infestare posse. —

Quare si genus febriculosum, de quo sermo est, a typho omnino distinguen dum esse post id, quod supra exposui, existimo, magis etiam, quam Griesinger feci in libro suo de morbis, qui nomine comprehenduntur *„Infectionskrankheiten,“* cu intermittenti conjungere id velim. Nam animo informare vix possum, quomodo mo

tribus circiter hebdomadibus constitutus, febris quaedam gastrica biliosa haberi
sit, post autem, illo tempore praeterito, natura intermittentis ejus appareat, maxime
um morbus, quod equidem sentio, a prima origine intermittentis formam et quasi
men in se habeat. Adeo quidem concedo Griesingero, viro magnae auctoritatis, ut
jecturam tantum proponam, quum quaeram, possitue forma intermittentis obscura
aedam atque obtecta diligentiore inquisitione investigari etiam quum stadium illud
sit acutum et gastroentericum, quod in superiore appellavi remittens? possitne
rte illa inveniri in acerbationibus febriculosis certis diei temporibus insistentibus? —
telligere non possum, quod Piorryus dixit, intermittentem propterea in remittentem
que continuam converti, quia gravioris cujusflam intermittentis paroxysmi vehementer
rogati inter se confundantur, et paroxysmorum subintrantium aut semper antepo-
ntium ordo quasi vinculo conjunctus remissionibus vix interrumpatur; — intelligere
n possum, hoc certis temporibus, casibus inumerabilibus simul apparentibus, iterum
saepius eventurum esse, quod tamen iisdem locis per magnum saepe annorum in-
rvallum ne semel quidem observatur. — Itaque rem ita mihi informavi, ut censeam,
t causam, ex qua malaria oriatur, si certa quaedam, sive locorum sive tempestatis
nditione sive alia quadam re, adhuc ignota, occasio oblata fuerit, praeter febrem
ermittentem ad producendum alium etiam morbum valere, in hoc casu ad efficien-
m gastroenteritidem, qua ratione fieri possit, ut ex ea causa cum intermittenti simul
ficiscatur gastroenteritis. Aut res ita se habet, ut iis temporibus, quibus malariae
sa sola dominetur, intermittens tantum exoriatur, quum alia sit causa ad produ-
dam gastroenteritidem necessaria, quae adhuc ignoretur. In utroque casu gastroen-
tis febris complicatio quaedam esset, febris autem marchiae, quae dicitur, inter-
tens quaedam cum gastroenteritide complicata. In qua sententia eo potius perseverare
im, quo magis videmus intermittentes ceteris quoque terris, dum procedunt, cum
ere gastrico ratione diversa complicatas esse. Itaque ad conclusioeem pervenio,
rem in Marchia grassatam inihil aliud esse nisi intermittentem vulgarem, eamque
magno morbi gastrici aut biliosi gradu conjunctam. Jam quid dicamus, si Han-
us, medicus Tonningensis anno scr[illegible]it 1735: „morbus Eiderostadenses corripiens,
aliquis morbus ex genere morbor[illegible] intermittentium, qui ex causa oritur poly-
lica?“ Recte illum dixisse puto et rectius etiam dixisset, si verbis usus esset his:
causa gastroenteritica, saepe cum affectione hepatis, imprimis polycholia compli-
et iis obtecta.“

Jam vero ca modestia, qua medidicum profiteri decet juniorem atque munus
licum primum adeuntem, eum morbum habendum esse censeo febrem, quam trac-
o mihi proposui, cui quum plerumque stadium prodromorum absit, stadium ga-
enteritidis acutae, per tres hebdomades manentis, naturam exhibeat febris remit-
is, ex qua febris intermittens excolatur manifesta et diversis formis certo apparens.
astroenteritidis loco alii morbi primum observantur, tum certe febris adest inter

mittens compliata, talis quidem, quae febri Marchiae simillima est. Attamen ut definitionem de hac ipsa febri institutam tuear neque perturbationem in his rebus ingentem augeam, febrem marchiae tum loco invasisse maxime affirmo, si gastroenteritis acuta febrem certe explicatam antecesserit. Nimirum signa gastrica aliaque in hoc primo stadio simul apparare possunt, quod si fit, eo numquam cohiberi nec possumus nec debemus, quominus febrem etiam illam, de qua dicimus, adesse putemus.

THESES.

1) In pneumonia hypostatica situs variatio est optimum remedium.

2) Resectio articulationis genu omnino non rejicienda.

3) Medicus plus noxiis removendis, quam remediis adhibendis, prodest.

THE INFLUENCE OF TEMPERATURE ON PLASMODIUM VIVAX

9

BY

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From the Laboratories of the International Health Division of the Rockefeller Foundation, New York

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INTRODUCTION

In this study of the influence of temperature on the sporogenous cycle of *Plasmodium vivax* in *Anopheles quadrimaculatus*, the following subjects are considered: (1) The temperature range in which the sporogenous cycle is completed, (2) the length of time necessary for the completion of the sporogenous cycle within the temperature range compatible with the exogenous development of the plasmodium, and (3) the effects of unfavorable temperatures at three periods of the exogenous cycle.

Although some of these subjects have been investigated in part by Grassi (1), Jancsó (2), King (3), Mitzmain (4), Roubaud (5), Wenyon (6), and others, opportunity for more extended studies has been afforded by the use of malaria therapy in paresis.

MATERIAL

Insectary-reared *A. quadrimaculatus* obtained from self-perpetuating colonies, established in an air-conditioned room, were employed as the test mosquitoes. The plasmodium utilized was the "McCoy" strain of *P. vivax*, which was isolated in Florida in 1931 and propagated by alternate transfer from mosquito to man for over 4 years without recourse to blood inoculation.

For the temperatures used in these experiments the following equipment was employed: (1) Two large incubator rooms kept at 37.5°C. and 28°C. constant temperature. (2) A small electric incubator adjusted to temperatures of 26, 30 and 32°C. (3) The air-conditioned insectary kept at 22°C., later changed to

24°C. (4) A Frigidaire regulated by thermostatic control fo
temperatures of 15 to 17°C. and below. These incubators varie
within 1°C. above or below the set temperature when recorded o
maximum and minimum thermometers. Thermographic record
showed that these variations from the set temperature were o
too short duration to be recorded by the thermograph.

TECHNIQUE

Control and experimental lots of anophelines were fed simu
taneously upon paretics undergoing malaria therapy who ex
hibited gametocytes of *P. vivax* in their peripheral blood. Ex
flagellation was always demonstrated before anophelines wer
applied. The mosquitoes were transported from the hospita
where they had fed on patients to the laboratory, where thos
which had taken a blood meal were separated from those whic
had not fed. The unfed mosquitoes were discarded. The tim
elapsing between the end of feeding and the placing of the mo
quitoes in the incubators was 1 to 1½ hours. During this perio
the mosquitoes were kept at approximately 28°C., a temperatur
favorable for the development of the plasmodium.

Infected mosquitoes were dissected at various stages of th
development of the exogenous cycle, and the salivary glands an
stomachs were examined. The stomachs were stained wit
brilliant cresyl blue and the oocysts were measured and counted.

RESULTS

The development of P. vivax *in* A. quadrimaculatus *when incubat at constant temperature throughout the exogenous cycle of the plasmodium*

Insectary-reared *A. quadrimaculatus* infected from gametocy
carriers of *P. vivax* were incubated at constant temperatur
ranging from 1 to 7°C. to 37.5°C. Table 1 shows that th
complete development of *P. vivax* occurred only at temperatur
between 15 to 17°C. and 30°C.

When anophelines which had taken an infecting meal we
incubated at a constant temperature of 37.5°C. no oocysts we
ever observed.

At a constant temperature of 32°C. no gland infection took place although, as judged by their size and morphology, the oocysts had developed to maturity on the eighth day of incubation at 32°C. The size of these oocysts was 45 to 50 micra; sporozoites could be seen within the oocysts, and when slight pressure was exerted on the coverslip to rupture the oocysts sporozoites were expressed. Examination of the oocysts in these mosquitoes 17 days after the infecting meal showed that the mosquitoes would never become gland positive, for only empty cyst capsules containing pigment were seen. It was demonstrated, however, that some of the oocysts were still viable on the seventh day of incubation at 32°C., for when part of the mosquitoes were removed to a temperature of 22°C., a scanty gland infection was observed to develop. A constant incubation temperature of 32°C. while it allowed the oocyst to develop to maturity, either caused the sporozoites to die within the oocyst or prevented their "migration" to the salivary glands after the cysts had ruptured.

TABLE 1

Effect of various constant temperatures on the development of the sporogenous cycle of P. vivax in A. quadrimaculatus

TEMPERATURE	NUMBER OF TRIAL LOTS	STOMACH DISSECTIONS			GLAND DISSECTIONS			DAYS REQUIRED TO COMPLETE SPOROGENOUS CYCLE
		Number examined	Number with oocysts	Per cent with oocysts	Number examined	Number with sporozoites	Per cent with sporozoites	
°C.								
37.5	1	30	0	0	18	0	0	Never completed
32	2	34	29	82	23	0	0	Never completed
30	2	18	13	61	11	8	78	8
28	5	55	50	91	44	32	73	8
26	1	7	7	100	1	1	100	10
23–24	6	63	59	91	70	54	79	12
22–23	8	80	72	90	90	80	89	14
15–17	5	46	30	65	9	9	100	38
13–18	1	11	0	0	9	0	0	Never completed
1–7	1	12	0	0	3	0	0	Never completed

No oocysts or sporozoites were found in *A. quadrimaculatus*

incubated at temperatures below 15 to 17°C. As dissections
the stomachs of these lots up to the eighteenth day of incubat
were negative, the mosquitoes were removed to the 22°C.
cubator to allow the oocysts, if any were present, to develop m
rapidly. However, no infected stomachs or glands were fou
during the 55 days the mosquitoes were under observati
Control lots (not shown in Table I) incubated at 28°C. showed t
the patients' blood was infectious for mosquitoes kept at favora
temperatures.

It is of epidemiological importance to note that adult
quadrimaculatus were able to survive a wider range of temperat
than the developmental forms of *P. vivax* which infect t
mosquito. If the humidity was sufficiently high the imagi
lived for a period of 2 weeks at 37.5°C. and withstood a tempe
ture of 1 to 7°C. for more than 2 months.

At whatever constant temperature the mosquitoes were
cubated (within the range of temperature compatible with t
development of the exogenous cycle), the resulting sporozoi
were infective. In other words, when the different lots
mosquitoes listed in Table I as having been incubated at consta
temperatures between 15–17°C. and 30°C. were applied
paretics, all patients developed verified clinical malaria.

Eight days was the shortest time in which *P. vivax* was observ
to complete its sporogenous cycle. This occurred when t
mosquitoes were incubated at 28°C. or 30°C. At 26°C. t
sporogenous cycle was prolonged to 10 days, at 23 to 24°C.
12 days, at 22–23°C. to 14 days, although an occasional lot
not become gland positive before 17 days at this last temperatu
Between the temperatures of 22 and 28°C. an increase of 1
usually shortened the time required for the completion of t
sporogenous cycle by 1 day. At lower temperatures the
cubation period was disproportionately prolonged, since at 15
17°C. the extrinsic incubation period was from 25 to 40 da
Within the temperature range compatible with the developme
of *P. vivax* in the anopheline the time necessary for the completi
of the sporogenous cycle decreased as the temperature increas

The effect on the development of P. vivax in anophelines which were exposed to high temperatures shortly after feeding

Anophelines having fed on a patient in the hospital were placed at temperatures of 37.5°C. on return to the laboratory. One to 1½ hours elapsed between the time when the anophelines had finished their blood meal and the time they were placed at 37.5°C. After varying periods of incubation at 37.5°C. the anophelines were removed to a favorable temperature of 20 to 24°C. The results are shown in table 2.

In the three lots of mosquitoes kept at 37.5°C. for 17 to 18 hours after feeding, none became infected. The average infection rate in the controls incubated at a favorable temperature was 90 per cent. None of the experimental lots showed sporozoites in the salivary glands, while the controls were gland positive on an average of 14 days.

In the second series kept at 37.5°C. for 2, 4, and 8 hours after feeding, none of the mosquitoes exposed to this temperature for 4 hours or longer become infected. Those kept at 37.5°C. for 2 hours were gland positive in 13 days, the same time as the controls. There was no significant difference in the infection rate of the controls and the experimental lot kept at 37.5°C. for 2 hours, but there was a higher percentage of heavily infected stomachs in the control lot than in the experimental lot.

In the third series three lots of mosquitoes were kept at 37.5°C. for 2, 3, and 4 hours after feeding. No plasmodial infection was found in anophelines kept at this temperature for 3 hours or longer. The infection rate in the lot kept at 37.5°C. for 2 hours was only slightly less than in the control lots, although 50 per cent of the control lots had heavier stomach infections as determined by oocyst counts. In the fourth, fifth, and sixth series the findings were in agreement.

When *A. quadrimaculatus* which had fed on a gametocyte carrier of *P. vivax* were placed at 37.5°C. within 1 to 1½ hours after feeding, the percentage of mosquitoes developing plasmodial infection decreased progressively the longer they were kept at this unfavorably high temperature. After exposure to 37.5°C.

for 3 hours only 3 per cent of the experimental lots became
fected as compared with an infection rate of 72.8 per cent in

TABLE 2

Effect of varying time of exposure at 37.5°C. on the development of the sporoge cycle in A. quadrimaculatus

SERIES NUMBER	NUMBER OF HOURS AT 37.5°C.	LOTS OF MOSQUITOES	STOMACH DISSECTIONS			GLAND DISSECTIONS			DAYS REQUIRED COMPLETE SPOR ENOUS CYCL
			Number examined	Number with oocysts	Per cent with oocysts	Number examined	Number with sporozoites	Per cent with sporozoites	
I	17	1	10	0	0	10	0	0	Never comple
	18	1	27	0	0	20	0	0	Never comple
	18	1	13	0	0	13	0	0	Never comple
	Controls*	3	19	18	95	84	71	84	14
II	2	1	12	9	75	6	6	100	13
	4	1	12	0	0	10	0	0	Never comple
	8	1	9	0	0	6	0	0	Never comple
	Controls*	1	9	7	78	10	9	90	13
III	2	1	12	10	83	4	1	25	14
	3	1	11	0	0	1	0	0	Never compl
	4	1	11	0	0	†			
	Controls*	1	12	11	92	24	15	63	12
IV	2	1	14	3	21	6	3	50	14
	3	1	13	1	7.6	4	1	25	14
	4	1	0	0	0	6	0	0	Never compl
	Controls*	1	7	6	86	5	3	60	13
V	1	1	12	6	50	6	0	0	Never compl
	2	1	14	4	29	8	1	13	16
	3	1	9	0	0	3	0	0	Never compl
	Controls*	1	10	7	70	5	0	0	Never compl
VI	1	1	14	3	21	6	3	50	14
	2	1	13	1	7.6	4	1	25	14
	3	1	15	0	0	6	0	0	Never compl
	Controls*	1	7	6	86	5	3	60	13

* Incubated at optimal temperatures of 20 to 24°C.

† Dissected before sporogenous cycle was completed in control lot.

controls. At 4 hours or longer at this temperature none of
experimental lots became infected.

The effect of high temperatures upon the developing oocysts of P. vivax in A. quadrimaculatus following incubation at favorable temperatures

Separate lots of *A. quadrimaculatus* infected with *P. vivax* were incubated at favorable temperature (22 to 24°C.) for 7 to 13 days after an infective feeding. To serve as a control a part of each lot was allowed to remain at this favorable temperature. The remaining mosquitoes of the same lot were placed at 37.5°C. for periods of 1 hour to 7 days, after which time they were again put at a temperature favorable for the development of the plasmodium. The results are shown in table 3.

TABLE 3

Effect on the development of the sporogenous cycle in A. quadrimaculatus when the mosquitoes were exposed to unfavorable temperature (37.5°C.) following incubation at optimal temperature (22 to 24°C.)

EXPOSURE AT OPTIMAL TEMPERATURE, 22–24°C.	EXPOSURE AT UNFAVORABLE TEMPERATURE, 37.5°C.	STOMACH DISSECTIONS			GLAND DISSECTIONS			DAYS REQUIRED TO COMPLETE SPOROGENOUS CYCLE
		Number examined	Number with oocysts	Per cent with oocysts	Number examined	Number with sporozoites	Per cent with sporozoites	
days	*hours*							
8	1	1	1	100	9	5	56	14
8	8	2	2	100	8	8	100	15
10	12	2	2	100	8	8	100	17
9	18	9	8	89	9	0	0	Never completed
9	24	10	8	80	8	1	13	21
10	24	4	3	75	9	0	0	Never completed
12	24	2	1	50	7	1	14	36
13	24	7	6	86	9	0	0	Never completed
10	36	10	8	80	10	0	0	Never completed
7	168	7	5	71	5	0	0	Never completed
Controls*		84	76	91	161	136	84	14

* Exposed at 22 to 24°C. throughout experiment.

Exposure of mosquitoes with developing oocysts to a temperature of 37.5°C. for 1 to 12 hours had no demonstrable effect in shortening or increasing the time for the completion of the sporogenous cycle. There was no deleterious effect on the oocysts as judged by the number of anophelines which became gland positive: 100 per cent of the anophelines placed at 37.5°C. for 8 to 12 hours showed sporozoites in the salivary glands on dissection. The first unfavorable effect of high temperature on the developing oocyst was observed in the lot which was exposed to 37.5°C. for 18 hours. Although 89 per cent of this lot showed oocysts on stomach dissection, none of 8 glands examined on the seventeenth and twenty-first days after the infective feeding showed sporozoites. Stomachs examined on these 2 days showed only remains of degenerate cysts.

In four lots of anophelines placed at 37.5°C. for 24 hours only an average of 6 per cent became gland positive, although the stomach infections of these lots were 50 to 86 per cent. Of the control lots 84 per cent developed sporozoites.

One lot placed at 37.5°C. for 36 hours never became gland positive, although 80 per cent of the stomachs of this lot had oocysts. One hundred per cent of the control lot became gland positive on the seventeenth day. One lot incubated at 22°C. for 7 days and at 37.5°C. for 7 days never became gland positive while it was under observation. Seventy-one per cent of the stomachs of this lot showed oocysts.

When the development of the oocyst was well advanced (7–13 days after the infective feeding), exposure of the infected anophelines to 37.5°C. up to 12 hours did not prevent the further development of the oocysts, but exposure to this unfavorable temperature for 18 hours or longer completely stopped the development of the oocysts in all but a very small percentage of mosquitoes.

The effect on the developing oocysts of intermittent exposures to an unfavorably high temperature

From the time of its infective feeding until it was found gland positive, one lot of mosquitoes was incubated at 28°C. except for 7 hours daily exposure to 37.5°C. Subjected to this method of incubation the mosquitoes were found to be gland positive on the sixteenth day after the infecting blood meal while the control lot, incubated at a constant temperature of 28°C., was gland positive in 8 days. There was no significant difference in the stomach infection rate or in the oocyst counts of the experimental lot and its control. However, the percentage of positive glands in the experimental lot was 33.3 per cent as against 88.8 per cent in the controls. The number of sporozoites found in the positive salivary glands of the experimental lot was very small, while in the control lot the sporozoites in the salivary glands were abundant.

It was demonstrated that intermittent exposure to unfavorably high temperatures prolonged the incubation period of *P. vivax*. Although there was no difference in the stomach infection rate or in the oocyst counts, a smaller number of mosquitoes became gland positive, and in these the number of sporozoites found in the salivary glands was scanty compared with the controls.

The effect of exposure to high temperature on the infectivity of A. quadrimaculatus after the sporogenous cycle was completed

Anophelines which had completed their incubation of *P. vivax* at a favorable temperature were exposed to 37.5°C. for 8, 16, and 24 hours. One patient inoculated with three gland positive mosquitoes kept at 37.5°C for 8 hours developed clinical malaria after an incubation period of 12 days; the controls developed clinical malaria on the eleventh and twelfth days after inoculation. Two patients, each inoculated with one gland positive mosquito which had been kept at 37.5°C. for 16 hours, developed clinical malaria after incubation periods of 14 and 15 days, their controls in 11 and 12 days. No difference was observed in the clinical course of the patients inoculated with the mosquitoes of the experimental lots and those inoculated with mosquitoes always kept at favorable temperature. Two patients were inoculated with gland positive anophelines which had been kept at 37.5°C. for 24 hours. The first patient inoculated with three such mosquitoes developed no clinical malaria; the second patient inoculated with six mosquitoes developed clinical malaria after an incubation period of 20 days and exhibited three tertian paroxysms followed by a spontaneous termination of the clinical attack. The control patient on whom only three control mosquitoes had fed developed clinical malaria after an incubation period of 10 days, experienced 16 quotidian paroxysms followed by a fever-free period of 7 days, then had a relapse of such severity that it was necessary to terminate the malaria attack by the administration of quinine.

The effect on the development of P. vivax when anophelines were exposed to low temperatures shortly after feeding

A. quadrimaculatus which had taken a blood meal on a patient undergoing malaria therapy were kept at a favorable temperature for 1½ hours in transit from the hospital to the laboratory. They were then placed at temperatures of 1 to 10°C. for periods ranging from 1 to 7 days. The results are shown in table 4. Plasmodial infection of the anophelines occurred in those lots kept at a low temperature for 2 days or less. None of the anophelines kept at 1 to 10°C. for 2½ days or longer developed stomach or gland infections.

TABLE 4

Effect of low temperature on the development of the sporogenous cycle in A. quadrimaculatus beginning 1½ hours after blood meal

EXPOSURE AT LOW TEMPERATURE		STOMACH DISSECTIONS			GLAND DISSECTIONS			DAYS REQUIRED TO COMPLETE SPOROGENOUS CYCLE
		Number examined	Number with oocysts	Per cent with oocysts	Number examined	Number with sporozoites	Per cent with sporozoites	
days	*°C.*							
1	3–6	4	4	100	7	7	100	16
2	3–6	4	3	75	7	6	86	16
2½	3–10	7	0	0	7	0	0	Never completed
4	1–6	9	0	0	9	0	0	Never completed
7	2–6	10	0	0	9	0	0	Never completed
Controls		14	12	92	117	95	81	15

The effect of low temperature upon the developing oocysts of P. vivax following incubation at favorable temperature

After feeding on a gametocyte carrier separate lots of *A. quadrimaculatus* were incubated at a favorable temperature (22–24°C.) for 7 to 10 days. To serve as a control part of each lot was kept at a favorable temperature throughout the development of the sporogenous cycle. Other mosquitoes from these same lots were placed at temperatures of 1 to 10°C. for periods of 1 to 35 days. The results are shown in table 5. The sporogenous cycle was usually completed in the lots which were kept at a low temperature for 21 days or less. None of the anophelines kept at 1 to 10°C. for longer than 24 days showed sporozoites in their salivary glands on dissection.

TABLE 5

Effect on the development of the sporogenous cycle in A. quadrimaculatus when the mosquitoes were exposed to low temperature (1 to 17°C.) following incubation at favorable temperature (22 to 24°C.)

EXPOSURE AT OPTIMAL TEMPERATURE, 22-24°C.	EXPOSURE AT LOW TEMPERATURE		STOMACH DISSECTIONS			GLAND DISSECTIONS			DAYS REQUIRED TO COMPLETE SPOROGENOUS CYCLE
			Number examined	Number with oocysts	Per cent with oocysts	Number examined	Number with sporozoites	Per cent with sporozoites	
days	*days*	°C.							
10	1	3–8	3	3	100	8	7	87	15
10	2	1	3	3	100	5	3	60	27
10	4	1	2	2	100	8	5	63	28
10	8	1	10	8	80	9	0	0	Never completed
8	14	1–8	7	7	100	8	5	63	30
8	21	1–8	5	5	100	15	5	33	37
8	28	1–8	11	11	100	5	0	0	Never completed
8	35	1–8	8	7	87	7	0	0	Never completed
7	2	2–8	12	10	83	12	10	83	18
7	7	2–8	8	8	100	12	10	83	24
7	15	2–8	13	9	69	5	4	80	43
7	24	2–8	4	3	75	14	4	29	43
7	31	2–8	14	4	29	9	0	0	Never completed
7	23	15–17	7	5	71				
Controls*			50	47	94	119	89	73	15

* Exposed at 22 to 24°C. throughout the experiment.

The effect of exposure to low temperatures on the infectivity of A. quadrimaculatus after the sporogenous cycle was completed

In our studies on induced malaria anophelines were routinely stored at 1 to 7°C. after completion of the sporogenous cycle when sporozoites were found in the salivary glands. At this temperature repeated trials showed that after 50 days at 1 to 7°C. the infectivity was lost as when these mosquitoes were applied to paretics, these patients did not develop verified clinical malaria.

SUMMARY AND CONCLUSIONS

1. The sporogenous cycle of *P. vivax* in *A. quadrimaculatus* was completed within the temperature range of 15–17°C. to 30°C.

2. The shortest time in which the cycle was completed in the insect vector was 8 days after the infective feeding; the longest period was 38 days.

3. The optimum temperature for the development of the exogenous cycle of *P. vivax* was 28°C., the lowest temperature at which the cycle was completed in the shortest time.

4. In all stages of the exogenous cycle, *P. vivax* was more susceptible to unfavorably high temperatures than to unfavorably low temperatures.

5. Shortly after feeding on a gametocyte carrier *A. quadrimaculatus* was completely sterilised of its plasmodial infection at 37.5°C. in 2 to 3 hours; at temperatures of 1 to 10°C. 2½ days were necessary. During the growth of the oocysts (7 to 13 days after the infective feeding) exposure of the mosquito to 37.5°C. for 18 to 24 hours aborted its development, only a very small percentage of these mosquitoes ultimately showing sporozoites in their salivary glands. At 1 to 10°C., 24 days were required to interrupt oocyst development. When the sporogenous cycle was completed and sporozoites were present in the salivary glands of the anophelines, 24 hours' exposure to 37.5°C. had a marked inhibitory effect on the infectivity of the sporozoites in human inoculation. This effect was not observed in anophelines kept at 1 to 7°C. until after 50 days at this low temperature.

6. The developmental forms of the malaria parasite present shortly after feeding were most susceptible to unfavorably high and low temperatures; the sporozoite was least suceptible to unfavorable temperatures; the oocyst occupied an intermediate position.

7. Anophelines which developed their malarial infection when incubated at the lowest or at the highest temperatures possible for the completion of the exogenous cycle were infective.

8. The fact that *P. vivax* will not develop at constant temperatures above 30°C. and the fact that a period of 24 hours at 37.5°C. will sterilize all but a very small per cent of *A. quadrimaculatus* of their *P. vivax* infection have a bearing on the transmission of *P. vivax* infection during the summer months. The data presented show that in certain localities the oocyst may survive the winter and complete its development the following spring.

9. The adult *Anopheles quadrimaculatus* will survive higher and lower temperatures than the developmental forms of the malaria parasite which infect this insect.

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ON THE DURATION OF INFECTIOUSNESS IN ANOPHELINES HARBORING PLASMODIUM FALCIPARUM

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BY

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ON THE DURATION OF INFECTIOUSNESS IN ANOPHELINES HARBORING PLASMODIUM FALCIPARUM[1]

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In our experience to date, the outcome of inoculations made with *Plasmodium falciparum* has been distinctly less certain than in the case of those made with *Plasmodium vivax*, even though in each case the presence of sporozoites in the salivary glands of the mosquitoes was verified after their application. In all except three instances the inoculations reported have been in negroes or colored persons. Failure cannot be attributed to immunity of the patients, as in every instance where an inoculation has been unsuccessful, we have ultimately, by using other mosquitoes

TABLE 1

TIME ELAPSING SINCE COMPLETION OF EXTRINSIC INCUBATION PERIOD	TOTAL INOCULATION WITH P. FALCIPARUM	TOTAL SUCCESSFUL INOCULATIONS	PER CENT SUCCESSFUL
days			
1–10	31*	26	84
11–20	39	28	71.8
21–30	11	3	27.3
31–40	11	1	9.1
41–50	6	0	0.0
51–60	0		
All...............	98	58	59.4

* Two failures following use of mosquitoes on first day gland-positive.

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[1] The observations on which this paper are based were made with the support and under the auspices of the International Health Division of the Rockefeller Foundation in coöperation with the Florida State Board of Health and the Florida State Hospital.

(usually infected with the same strain), invariably succeeded in infecting the patient. The uncertain results cannot therefore be credited to qualities of the patients.

After the mosquitoes have matured their infection with *Plasmodium falciparum*, they are transferred for storage to a Frigidaire kept at a temperature ranging from 3° to 14°C.

Since the unsatisfactory results appeared attributable to the mosquitoes rather than the patients, we were led to analyze our experience with the different lots according to the time elapsing between the termination of the extrinsic incubation period and the employment of the mosquitoes for inoculation. The results are presented in table 1.

During the first ten-day period the results have been quite satisfactory, 84 per cent of all inoculations taking, and if we exclude 2 patients inoculated with mosquitoes that had become gland-positive on the same day, 90 per cent of inoculations with mosquitoes in this period have been successful. Thereafter, however, the effectiveness of the mosquitoes rapidly diminishes. We have not succeeded in any inoculation attempted with mosquitoes when more than forty days has elapsed since the completion of the extrinsic incubation period.

In this connection it is interesting to note that the infectiousness of mosquitoes harboring *Plasmodium falciparum* deteriorates much more rapidly than does that of those harboring *Plasmodium vivax* (1).

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STUDIES ON BENIGN TERTIAN MALARIA.

8. OBSERVATIONS ON SPLENOMEGALY.

11

By

WARREN K. STRATMAN-THOMAS.

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STUDIES ON BENIGN TERTIAN MALARIA.

8. OBSERVATIONS ON SPLENOMEGALY.* †

BY

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The observations presented in this paper are based on the routine examinations of the spleens of adult white male patients experiencing a single, primary, therapeutic infection with *Plasmodium vivax*.

Methods.

The patients to receive malaria therapy were selected by the regular medical staff of the hospital. Inoculation with *Plasmodium vivax* was effected by means of anopheline mosquitoes. The examinations were made at weekly intervals from the time of inoculation until the patient was discharged from the malaria service. Palpation for the detection of spleen enlargement was performed while the patient lay in a recumbent position with the knees flexed. The degree of enlargement was described as along a line drawn from the left costal margin through the umbilicus to the right iliac spine. The amount of splenic enlargement was classified according to the method of Boyd, which is as follows:

N.P.	Spleen not palpable during deep inspiration.
P.D.I.	Palpable only on deep inspiration.
No. 1.	Palpable on the costal border during normal inspiration.
No. 2.	Palpable, extending anywhere beyond the outer limit of the previous class half way to the umbilicus.
No. 3.	Palpable, extending to the umbilicus.
No. 4.	Palpable, extending beyond the umbilicus.

---

[*] The observations on which this paper are based were made with the support and under the auspices of The Rockefeller Foundation, in cooperation with the Florida State Board of Health and the Florida State Hospital.

[†] Read before the National Malaria Committee, meeting at Richmond, Virginia, November 17, 1933, in joint session with the American Society of Tropical Medicine.

Material.

Data from fifty-eight cases are presented. Other cases are not considered, as palpation was unsatisfactory because of a very adipose or muscular abdomen, or because the patient would not cooperate with the examiner.

Results.

The fifty-eighth cases were divided as follows into four classes according to the duration of their clinical attack:

I. Duration of 10 days or less, 21 cases.
II. Duration of 11 to 20 days, 21 cases.
III. Duration of 21 to 30 days, 10 cases.
IV. Duration of over 30 days, 6 cases.

The spleen measurements of the patients in each of these four classes were tabulated separately by weeks elapsing after the onset of the attack of clinical malaria (taken arbitrarily as the first day that the temperature reached 100° F.).

Arbitrary numerical values representative of proportional increase in splenic volume were assigned to the various classes for the purpose of computing the average spleen, as follows:

Not palpable	1
Palpable on deep inspiration	2
Number 1 spleen	3
Number 2 spleen	5
Number 3 spleen	8

The size of the average spleen during successive weeks of illness in attacks of differing duration is shown in table 1. Patients who suffered a relapse of verified clinical malaria are separated from those who did not have a relapse.

Discussion.

Cases not relapsing. In table 1 it is seen that the degree of splenic enlargement attained is directly proportional to the duration of the clinical attack. Attacks of 10 days' duration or less tend to promote palpability only on deep inspiration, with the maximum enlargement noted at the end of the second week. This enlargement disappears during the third week after the clinical attack. Patients experiencing clinical attacks of from 10 to 20 days' duration tend to develop as a maximum a number 1 spleen, which occurs during the second week of the clinical attack. After the sixth week the spleen tends to de-

TABLE 1.

Size of average spleen during successive weeks of illness in attacks of differing durations.

Non-relapsing cases	1st week	2d week	3d week	4th week	5th week	6th week	7th week	8th week	9th week
10 days or under (18 cases)	1.29	1.41	1.23	1.44	1.44	1.0	1.0		
11 to 20 days (18 cases)	1.58	3.07	3.11	3.08	2.55	3.11	1.87	2.0	
21 to 30 days (10 cases)	1.14	2.30	4.0	4.2	3.66	2.6	1.77		
Over 30 days (5 cases)	1.40	4.0	5.0	5.0	5.4	4.75	4.0	3.0	
Relapsing cases									
10 days or under (3 cases)	1.0	2.0	2.66	3.33	5.0	4.0	5.5	3.0	3.0
11 to 20 days (3 cases)	1.0	2.0	3.0	4.0	6.0	6.0	3.5	5.0	
21 to 30 days (no relapses)	—	—	—	—	—	—	—	—	—
Over 30 days (1 case)	1.0	2.0	5.0	8.0	8.0	8.0	8.0	8.0	

crease in size until it is palpable only on deep inspiration. Patients experiencing attacks of 21 to 30 days' duration tend to have a number 2 spleen as the maximum degree of splenomegaly, which occurs during the third week after onset. By the fifth week the spleen has decreased to number 1 dimensions and in the sixth week it may be only palpable. Eight weeks after onset the splenomegaly has usually disappeared. Patients experiencing a clinical attack of more than 30 days' duration tend to develop a number 2 spleen (slightly larger than that of the previous group) as a maximum, during the second week, although the spleen continues to increase in size until the fifth week. It may persist in this degree until the seventh week, after which the enlargement begins to decrease.

Case relapsing. The relapsing cases are too few in number to permit of any definite statement, except that while the maximum splenic enlargement persists there appears to be the possibility of a relapse.

Conclusions.

1. The degree of splenic enlargement attained is proportional to the duration of the primary clinical attack.

2. After the cessation of the clinical attack the spleen quickly tends to decrease in size.

3. If the clinical attack subsides but the maximum degree of splenomegaly persists there is possibility of a relapse.

4. Since splenic enlargement is transitory, spleen surveys in the field should be made during the height of the malaria season.

Dr. Hayden visited the locality of Mr. Lucas' excavations, and informs me that the formation from which the *Camarasaurus* was obtained, is the Dakota. Prof. Marsh has attempted to identify what is, according to Prof. Mudge, the same horizon, one hundred miles north of Canyon City, with the Wealden of England. Specimens from the northern locality which I have examined render it certain that the horizon is that of Mr. Lucas' excavations. Of this I may say that there is no palæontological evidence of its identity with the Wealden. The resemblance of the vertebrate fossils to those of the English Oolite is much greater, but not sufficient as yet for identification.

The discovery of *Vertebrata* in the strata of the Dakota epoch is an important addition to the geology and palæontology of North America. Credit is due to Superintendent O. W. Lucas for this discovery, and also in an especial manner for the skill and care he has exercised in taking out and shipping the ponderous specimens.

——:o:——

THE DISCOLORED WATERS OF THE GULF OF CALIFORNIA.

BY THOS. H. STREETS, M.D., U. S. N.

ONE of the earliest names given by the old Spanish navigators to the body of water that lies between the peninsula of Lower California and the western coast of Northern Mexico was the Vermilion sea. It was also known in the earlier times as the Mar de Cortez, and Mar Laurentano; or the Mar Vermiglion, Mar Rojo, and Mar Vermijo, on account of the reddish color of its waters; and more recently as the Mar, or Gulfo, de California.

The names Vermiglion, Vermijo, and Rojo seem to have been applied as early as between the years 1537 and 1540, after the explorations of Ulloa and Alarcon, and from the accounts given of it by Nuño de Guzman and his officers, who were the conquerors and rulers of Sinaloa, a state bordering on the gulf.

In all these narratives, however, it is well to note that two entirely distinct causes of discoloration are confounded. Father Consag and Ugarte, in particular, speak of the brick-colored corrosive water of the gulf-head, which is altogether different from the more vermilion-colored patches at the mouth of the gulf, which were, doubtless, what suggested the name Vermilion sea.

J. Ross Browne, at a recent date, and before him De Mofras, discredited the statements of the old Spanish sailors, and attributed the red color to the reflection in the water of the brilliant, rosy-tinted clouds of sunset.

We had ample opportunity to verify the truth of the Spanish account during the extended survey of the gulf and its shores by the U. S. Steamer "*Narragansett*," in 1875.

We first saw patches of the red water south of Ciralbo island. The steamer was stopped and a bucket was thrown overboard to secure some of it for examination. The water thus procured revealed nothing when subjected to the test of the microscope; it was perfectly transparent. The failure to discover the cause in this instance was due to the fact that the coloring matter does not float upon the top of the water, but is suspended several feet below; and when this was ascertained, by putting a lead in the bucket and sinking it a half fathom or more below the surface, there was no difficulty in obtaining all that was desired.

When first drawn up and viewed in a glass vessel by the unaided eye the water had a faint reddish tinge. When allowed to stand for a half hour the coloring matter settled to the bottom of the vessel as a greenish-yellow precipitate; and when some of this was taken up by a pipette and examined under the microscope, it was seen to be composed of minute roundish bodies. Further on these were proven to be the remains of ciliate infusoria.

The latter fact was not discovered, however, until after much painstaking investigation. Some small objects had been seen repeatedly to dart across the field of vision when the water was placed fresh upon the glass slide, but they disappeared as they came, like a flash, and it was a long time before their disappearance could be satisfactorily accounted for. The molecules were the only bodies that were permanent, and I was inclined to attribute to them the phenomenon of the water. Finally, one of the little bodies mentioned above stopped directly in the centre of the field of vision, and commenced a rapid rotatory movement, which presently ceased, and the animal was quiescent for a second or two; when lo! a rupture occurred, its molecular contents oozed out, and its transparent envelope became invisible. This then was the solution of the mystery. Afterwards, knowing what to search for, there was no difficulty in seeing the same process repeated again and again.

Mr. Darwin in his "Naturalist's Voyage Around the World," encountered a patch of similarly discolored water off the coast of Peru. According to his account, the water when examined by the microscope was "seen to swarm with minute animalculæ darting about and often exploding. Their shape is oval and contracted in the middle by a ring of vibrating curved cilia. It was, however, very difficult to examine them with care, for almost the instant motion ceased, even while crossing the field of vision, their bodies burst." From the foregoing it is evident that the water of the two localities is discolored alike by the same cause.

Those from the Gulf of California exhibited the same rapid to and fro motion as the ones seen by Darwin, and this motion was succeeded by a rotatory movement on the longer axis. Sometimes the rupture took place as soon as the latter motion ceased, and at other times the animal was motionless for a few moments preceding its final dissolution; and it was during these intervals of quiescence, only now and then observed, that anything like a correct impression of its general outline and structure could be gained. The following is the result of numerous observations extending over considerable time. The animal is oval in outline, with a projecting lip at its broad extremity, fringed with cilia. The rotatory movement took place around the smaller end as a pivot, and it advanced the same end in its to and fro movements. The envelope is a transparent and, apparently, structureless membrane, and in its interior are greenish-yellow granules, or bodies with dark rims and bright centres. These bodies floated loosely in the cavity of the animal, for when it revolved they changed their places like pebbles in a revolving cask. They averaged about the 1–12000 of an inch in diameter.

According to Mr. Darwin the bursting, which generally occurred at the extremities of the body, is due to an expansion of the tegumentary covering. I can not reconcile this with the results of my own observations. On the contrary, I noticed a contraction to take place in the transverse axis of the body; its length was increased and its transverse diameter diminished; in other words, the oval form was replaced by an oblong. This will better illustrate why the rupture generally took place at one of the extremities of the body. If these observations are correct the bursting of the envelope would be the result of an active rather than a passive action. The only reasonable way of accounting for an expansion of the body-covering would be by an imbibition of water from the outside, and a rupture would as likely occur in one place as in another in such a case.

When confined in a drop of water, or in an animalcula cage, the little animals never lived as long as a minute, the field of vision was often strewn with their remains before the observer had time to look through the tube of the microscope. This was especially the case if some time had elapsed since their removal from the sea. When kept in a bucket or in any vessel containing a large quantity of water they lived for a much longer time.

Many experiments were made with destructive agents with the view of killing the animalculæ before they had time to destroy themselves, but the results were unsatisfactory. In every instance they failed to accomplish the end for which they were used; if they served any purpose it was to accelerate the process of self-destruction.

The only explanation that I can give for this suicidal propensity lies in the abstraction of oxygen from the water; yet this appears somewhat exceptional in view of the fact that this gas is rather immaterial to the existence of the low, structureless forms of animal life. The bacteria, for instance, will flourish in infusions that have been boiled and hermetically sealed.

We have a record of these minute animals having been in this locality for more than three hundred years, defying the combined action of the winds, waves, and currents, and remaining as closely aggregated as a community of individuals endowed with reason or instinct, and not exposed to dispersing causes. They apparently have great control over their movements, at least so far as regards the fixity of their positions.

The great Colorado River, at the head of the gulf, constantly pours into it an immense volume of water which has a tendency to carry things seaward, as it does logs and other drift; yet here is this microscopic animal, the 1-1000 of an inch long; exposed to the same influence, but remaining in its chosen locality for centuries. What keeps these masses together forever in one place in spite of the circulation of the waters on the surface of the earth? Well might the question have presented itself to the mind of Darwin, but his fertile imagination suggested no adequate solution for it.

Other patches of discolored water obey the same impulse. In

1835 Mr. Darwin investigated that off the coast of Peru. The earliest notice we have of its existence in that locality is the beginning of the nineteenth century, and a good authority might be cited for its existence there as late as 1872. In the latter year while sailing down the eastern coast of South America we passed through a tract, while off the mouth of the Rio de la Plata, where the surface of the water was covered with a thick scum, resembling saw-dust. Darwin, in 1832, or forty years previous, found it further north, in the vicinity of the Abrolhos Islets. In this case the material that discolors the water belongs to the vegetable kingdom. It is a "minute cylindrical conferva," the *Trichodesmium erythræum*. According to Ehrenberg, the color of the water of the Red Sea is due to the presence of a "peculiar genus of alga," and Darwin states, on another authority, that it is the same species as that found off the coast of Brazil. In the latter locality it would certainly argue a defect of the vision to call the water red. Old sailors call it whales' feed, which, with them, is the generic title of everything that discolors the water of the ocean.

"The brick-colored and corrosive waters of certain parts of the gulf-head" received an easier solution at our hands than the more vermilion patches of the mouth of the gulf. The former is spoken of as being so extremely caustic as to remove the skin from the body like a blister, and to cause obstinate boils and ulcers, similar to those produced by scurvy, and which lasted for a long time. Some have gone so far as to even state that it would rot the clothes of those who were incautious in meddling with it. A recent compiler has endeavored to account for this peculiar property of the water on the supposition of an "excess of the iodides, bromides and sulphurets of minerals, derived, doubtless, from the abundance of volcanic material so common in these portions of the gulf." But as is common in such cases of explanatory guessing, his answer is not the true one.

Most of our information concerning this water has come down to us with the accounts of the voyages of Father Consag and Ugarte, both of whom confined their explorations, in these parts, to the head waters of the gulf; the former in 1746, and the latter in 1721.

We encountered this peculiar caustic water in about the same locality assigned it by Consag and others. In the Bay of Muleje, an indentation in the coast more than half way up the peninsula, the whole surface of the water was of a milky-red color. The body that gives to this water the strangely caustic properties is not a mineral, but an animal—a flagellate infusorium—the common *Noctiluca miliaris.* In this well-sheltered bay they accumulate from their light specific gravity at the top of the water. How thick the stratum was we did not ascertain, but we may form some idea of its extent from the fact that we steamed through the tract in a straight line for four or five hours at a speed of about five knots per hour. We dipped a canvas bucket in their midst, and when the water drained off it remained half filled with the animalculæ. They resemble minute grains of boiled sago. Every drop of water was literally crowded with them. They were so small that it required two or three to cover the area of a pin's head. It is very easy to comprehend how, if this bay were agitated by the slightest cause, it would glow as a broad sheet of living fire.

The Spanish sailors bathed in this water, and according to their chroniclers their bodies were covered with boils and ulcers in consequence. They were only half-way right when they attributed their infirmities to the water. The lashes of the little *noctiluca* were, undoubtedly, the exciting agents—the direct cause of their troubles; but that they were insufficient, in themselves, to account for the severity of the symptoms the evidence of our own men under different conditions of bodily health sufficiently proves. That their ulcers were similar to the effects of scurvy they recognized; and that a scorbutic condition of the blood played an important part in their production is evident. All sailors in those days were more or less the subjects of scurvy, which was a greater obstacle to the spread of commerce than were the small crafts of the navigators. It is a disease that often manifests itself in the form of boils and ulcers, and it exaggerates all trivial bruises and injuries of the skin. If our own sailors had been scorbutic when they bathed in the water the same train of symptoms would undoubtedly have followed; and, as it was, the skin, in places, became considerably inflamed and swollen, but the only disagreeable symptoms were the burning and tingling that accompanied the inflammation, which was of short duration.

Strickland, C.
& Chowdhury, K. L.

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ABRIDGED
REPORT ON MALARIA
13
IN THE
ASSAM TEA GARDENS

With Pictures, Tables and Charts.

By
C. STRICKLAND, M.A., B.C.,
Professor of Medical Entomology,
School of Tropical Medicine and Hygiene, Calcutta.

Assisted by
K. L. CHOWDHURY, M.B., D.P.H.,
Malariologist.

And in Collaboration with

J. DODDS-PRICE, M.R.C.S., L.R.C.P.,
The Salana Tea Co., Ltd.

C. E. P. FORSYTH, M.D., D.P.H.
Tezpur District.

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Parasitic Protozoa

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* *This report is abridged from the original sent in to the Indian Tea Association. The Appendices mentioned in the text hereafter are filed separately for reference in the office of the Association.*

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ABRIDGED
REPORT ON MALARIA IN THE ASSAM TEA GARDENS.

PART I.

By

C. STRICKLAND, M.A., B.C.

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INTRODUCTION.

Genesis of the Enquiry.

THE enquiry into Malaria in the Assam Tea Gardens of which the following pages is the report was the outcome of the generosity of the Indian Tea Association which placed sufficient funds at the disposal of Lt.-Col. Megaw, I.M.S., as Director of the School of Tropical Medicine, to enable the investigation to be financed.

Primarily the suggestion for it came from Lt.-Col. McCombie Young, I.M.S., who in his report as Director of Public Health, Assam, for 1922 wrote as follows :—

'Beyond these observations no formal malaria research work has been conducted and the most striking aspect of our ignorance of Assam malariology is to be found in the fact that notwithstanding the enormous sums of money invested in the tea industry and the size of its labour force, the efficiency of which depends very largely upon the standard of health which is maintained in it, no precise scientific observations have been made as to what are the conditions which at times and in places tend to produce an intensive prevalence of malaria on tea estates. It is not beyond the bounds of probability that if only a fraction of the money which is now spent in the prophylactic administration of quinine to the labour force were to be spent in abolishing or limiting the breeding grounds of carrier mosquitoes (did we but know them), the expenditure would yield a handsome return in an increased efficiency of labour forces. Since a Government agency for this investigation is not likely to be available, one would suggest that great benefit to the province and to the tea industry might result if the energies of the Calcutta School of Tropical Medicine were to be directed towards this virgin field of endeavour. If the tea industry could be convinced of the probable advantages to be derived from such enquiry, the funds necessary for its prosecution would in all probability be readily available, and the undertaking of such work is one of the main objects for which the Tropical School was founded. Instead of repeating at a particularly inopportune moment, proposals for malaria research to be financed by Government revenues, one ventures to advance these proposals as being more appropriate to present conditions.'

Government of Assam accepted the recommendation in G.R. 2445 E., dated 18th June 1923, as follows :—

'It is obvious, however, that more is needed and the Government and their Minister would be glad if steps could be taken to follow up the Director's suggestion that the energies of the Calcutta Tropical School of Medicine might be directed to malaria research work in Assam, and that the tea industry would receive in the increased efficiency of their labour force a good return on any money which may be allotted to the School for such work.'

Col. Megaw and the Indian Tea Association then simultaneously took the matter up with the financial result already stated and I was detailed for the work, the sequel showing that Col. McCombie Young's demand for an enquiry was justified.

The advantages of a mosquito malaria survey.

The lines along which anti-malarial work is usually conducted are those comprising what is commonly called a mosquito malaria survey. The advantages of such are many and I would repeat what I stated on the subject at the 1925 Annual Meeting of the Assam Branch of the British Medical Association. What was said cannot be repeated often enough, the gist of it being accepted by nearly every authority on the subject: but the public lags behind; it cannot understand why any differential treatment should be meted out to anophelines as distinct from culicine mosquitoes, let along a differential code of etiquette for the knock-out of the forty or so species of anophelines. "While a malaria-prevention campaign without a previous survey can indeed be carried out by employing certain measures which are of general application, a survey has at least these three advantages; it enables one to :—

(1) find out if a healthy site be available;

(2) localise in unhealthy places the areas in which prophylactic measures should be adopted; and

(3) choose the most appropriate measures to deal with the situation."

"The third item is very important because some anti-malarial methods not being of general application might, if tried without knowing what situation is being dealt with, cause worse trouble; consider, for instance, open earth-drainage: one drains a swamp and eradicates the species *umbrosus*, and one introduces *maculatus* or *funestus*. In this connection Boyd says 'All anopheline mosquitoes are not carriers of the malaria parasites, and gross variations with regard to their breeding-habits are found among the different species of anophelines.' Thus, it often happens that in a mosquito-infected district where malaria is occurring, only a small portion of the total mosquitoes are of the carrier type, and these may have quite specialised breeding habits, so that general measures of mosquito destruction might miss them altogether. The first essential in any district is. therefore, to make a critical survey to ascertain what mosquitoes are carrying the parasite and what their breeding habits are; after which, if feasible, steps specifically directed against the carrier species may be undertaken. To illustrate the diversity of breeding habits the following instances may be quoted :—*Anopheles maculipennis*, a well-known carrier, breeds chiefly in swamps and marshes and does not breed in running water so that it can be attacked by draining the swamp. Conversely, *A. maculatus*, which gave rise to much trouble in the Federated Malay States, breeds in running water. Other things being equal, therefore, draining by open drains (the usual method) would not abolish but rather encourage the breeding of this species. Yet another, *A. stephensi*, is almost exclusively a well-breeder and can only be countered by screening or oiling the responsible wells. It can thus be seen that haphazard measures are almost bound to involve a waste of time and money, and may even render the last state of affairs worse than the first by taking away conditions under which harmless mosquitoes were breeding and replacing them by others suitable to the propagation of carrier species" (1924).

"On the same subject Sir Ronald Ross at a meeting of the Royal Society of Tropical Medicine (1924) stated how different species of mosquitoes must be dealt with in different ways. He said 'We must deal with each species on its merits,' to which dictum Major Austen agreed and Dr. Balfour and Major Austen both were of the opinion that it was necessary to make careful biological investigations before starting upon any anti-malarial campaign; while Watson (1924) said, 'Let me candidly admit that for some years we cleaned up the ravines in our hill-land in Malaya making the malaria worse. We had to adopt an entirely different method. Let me remind you, too, that in 1901 when the Federated Malay States Government gave me the money to clean up Klang, Dr. Braddon applied for money to clean Seramban, a town on the main range. Presumably he would have felled jungle and 'trained' streams, and would have caused a big outburst of malaria in, at that time, a healthy town. Had that occurred I can imagine the progress of malaria prevention in the Federated Malay States would have been seriously retarded as it has been in India by the experiment done in Mian Mir.' "

"The fact that Watson started to preach this doctrine of the specific prevention of malaria in 1911 and that it is still necessary in 1925 for the Society of Tropical Medicine to reiterate it seems rather lamentable."

To develop then the greatest advantage in malarial operations, it will have been seen from these remarks how important it is to do a survey previous to taking any action which otherwise may prove useless and certainly would be wasteful.

Previous Surveys in Assam.

From time to time other surveys than that which is the subject of the present paper have been conducted in Assam. Not all of them have been in tea-garden tracts, but an analysis of some of the data collected by their authors in conjunction with the material collected in the present survey has been included in this paper because of the general similarity of the terrain described with that of the tea gardens.

The most important of other surveys have been those at Lumding Junction by many Medical Officers in co-operation, a report having been published by Lt.-Col. T. C. McCombie Young, I.M.S. (1921); at Nalbari, Kamrup District, reported by Lt.-Col. S. R. Christophers, I.M.S. (1922); Lt.-Col. McCombie Young, I.M.S., and Captain B. S. Challam (1923); at Pasighat by Lt.-Col. McCombie Young, I.M.S. (1921); and lastly, at Doom Dooma by Sir Malcolm Watson (1924). The general paucity of malarial epidemiological work in Assam referred to above by McCombie Young must be admitted.

With regard to India in general, an estate medical officer writing to me said, 'Does it not strike you as most extraordinary that anti-malarial work has been so little thought of in India...... certain tea-garden doctors in Assam are trying to do things little by little' and the doctor goes on to cite an instance of general ignorance in the matter where a malarious though otherwise excellent bungalow was dismantled rather than look for breeding-places of the mosquitoes which were the cause of the trouble.

Present Survey.

With a view to obtaining the benefits of a survey as outlined above:

(1) to find if a healthy site be available;

(2) to localise the area in which measures should be undertaken;

(3) to choose the most appropriate measures for each situation;

we have studied:—

A. the variation in the amount of malaria in any given locality at different times, e.g., the seasonal incidence of fever cases, (*see* Part III below).

B. the variation in the amount of malaria in different places correlated with different features of the land, such as tanks, streams, etc. (*see* Part IV below).

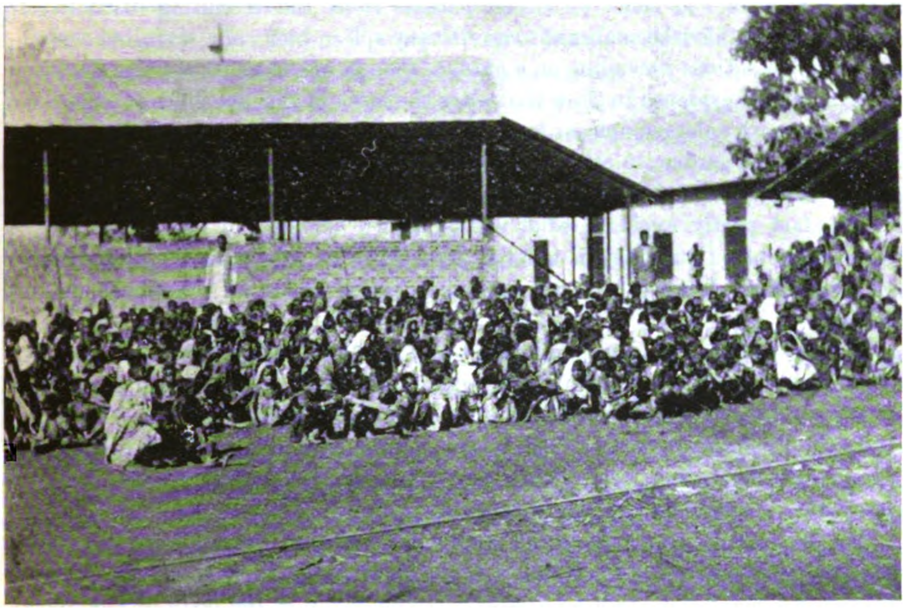
But how does one study 'the variations in the amount of malaria.' To answer this a short account, under the heading of *malaria indices*, of some means of measuring the amount of malaria in a place will now be given, and this will be followed by notes on what may possibly be important factors (including a reference to the meteorological data) in the surroundings.

Indices for the survey of the amount of malaria in a place.

(*a*) *The 'miscarriage' index.*

No figures for this event in Assam were collected but a series would no doubt be very instructive. If 'maternity benefits' on estates be widened in scope, perhaps such figures would arise.

'Immune' coolies apparently (*see* Dr. Murphy below) can show a very high birth-rate. As malaria sickness so commonly produces abortion, one must assume that the fever and not the infection does so.



[*Photo by Mr. J. Reid.*]

FIG. 1.—Cooly children on Alinugger Tea Estate. About 1,000 hostages to malaria.

(*b*) *The birth index.*

No data for this index on estates have been collated, (*see* in Part II Dr. Dodds-Price's article on the birth-rate of certain estates, and also Dr. Murphy's note referring to a garden with a very high spleen-index where the birth-rate was 58 per mille).

On Alinugger Estate in Sylhet the experience was that until in 1917 a good lot of presumably 'immune' 'jungly' coolies from Ranchi had been put on to the Estate, it was unhealthy and but few children could be raised. Since 1917 the result is expressed in the accompanying photograph (Fig. 1).

(c) *The infantile mortality index.*

This is a very good index of the amount of endemic malaria on an estate, and it ought not to be difficult with a slight adaptation of the existing system of maternity benefits to obtain very valuable records from the point of view of malaria prevention.

Dr. Ramsay is of opinion that in Cachar infantile convulsions are chiefly due to malaria and malaria is the principal cause of the general infantile mortality.

(d) *The spleen-index.*

It seemed important to test the point whether the spleen-index is subject to seasonal or ephemeral influences, as it is used so often for the comparison of malariousness in a locality at different times.

In the Tea Gardens Survey the index was culled from children from about the ages of 2 to 10. A note was made as to whether the children had been born on the garden or had come with their parents from the recruiting districts. It would, of course, have been absurd to ascribe any spleen-index of a recruited community to the conditions prevailing on a garden, though one of us (C. S.) has elsewhere collected certain evidence to show that after the lapse of 2 years children who come to a garden with enlarged spleens lose or retain them according to the healthiness or otherwise of a garden, in other words after a certain time *children from the country have the same spleen-index as those born on a garden.*

This fact partly explains, what will be shown hereafter in Part III, that the spleen-index of either class in a large area taken at any time, or alternatively that of a small locality over an extended period is for all practical purposes the same.

However, on tea gardens the locality was not large enough nor was the spleen-index in any lines taken often enough to justify reliance being placed on any figures but the comparative data obtained in only the garden-born children.

In the Preliminary Survey * the spleen indices were nearly all taken by myself or under my immediate supervision, (*see* Appendix IA),† while in the Detailed Survey the Sub-assistant in charge was responsible.

For the purpose of checking the latter, the index in certain lines was taken from time to time either by the V. M. O. of the Estate or by myself, and comparisons of the values obtained indicate that the figures given in the body of the report are not generally high enough (*see* Appendix IB).

The possibility of kala-azar confusing the malaria spleen-index was borne in mind and some observations made by Dr. Dodds-Price in collaboration with myself on one of his previously highly infected kala-azar gardens have since been reported (1925). They showed that unless anyone with an enlarged spleen could be at sight diagnosed as a kala-azar case, the condition could be ascribed to malaria, and if kala-azar were diagnosed the case should be excluded from the record.

For the whole of Assam the following spleen indices were obtained.

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* See page 17 under *Means and methods.*

† This Appendix like all the appendices is filed for reference in the office of the Indian Tea Association, Calcutta.

A. In the Preliminary Survey (1923)—
(*see* Appendix IA).

B. In the Detailed Survey (1924)—

Total number of all children examined	[*] 16,832
Number with spleen enlarged	7,352
Index	43·6%
Total number of G. B. children only	14,005
Those with enlarged spleen	6,077
Index	43·3%
Total number of imported children	2,827
Number with spleen enlarged	1,275
Index	45·01%

(*e*) *The hæmoglobin index.*

This index was taken in the same series of children as the parasite index; Tallquists' scale was used. The results are shown in Part III.

(*f*) *The parasite index and the species of parasite rate.*

This was not taken in the Preliminary Survey, but during the Detailed Survey it was arranged that it be taken systematically from 50 children a week giving about 5,000 slides in the year.

During the year the following were the results:—

Total children examined (good films)	1,520
Films with parasites	189
Index	12·4%

A too large proportion of the films prepared in the field were so bad that they could not be examined, and delay in the inception of the work occasioned further loss in the estimated numbers to be examined.

(*g*) *The malaria sickness index.*

This index is no guide to the malariousness of a garden. The garden may show a high spleen-index, which always truly betrays the malariousness of a locality, and yet the health may be very good. Dr. R. Murphy in a letter says: 'One of my healthiest gardens has a birth-rate of 58 per 1,000, deaths 18 per 1,000 and a spleen-index of 91 per cent.' Everyone knows that to obtain a high sickness rate on malarious estates one has only to bring in a batch of new coolies such as 'yellow-bellies' or Paharias from non-malarious localities and an epidemic among them will start immediately.[†] 'Jungly coolies like Oraons, Mundas, Santhals or the old coolies of the estate are "particularly immune" and do not get much sickness' (*see* Dr. Forsyth's article in Part II).

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[*] Examination of 30,000 was contemplated, but the slow development of the operations due to causes adverted to elsewhere as well as other reasons led to the above result.

[†] The importation of large numbers of 'unsalted' new coolies, i.e., if they are imported from non-malarial districts, means that a large percentage die and that the remainder more or less suffer from malaria and its effects until they become acclimatised. Until they become 'acclimatised to malaria' these new coolies more or less live on the hospitality of the Tea Estates and hence in my opinion it is much cheaper to look after the established labour force and breed from 'Immunes' (Dr. Ramsay).

The comparative sickness incidence then between different localities, or from time to time in one locality, is therefore no real index as to their comparative malariousness.

But if one takes these data over sufficiently wide areas, or over sufficiently long periods of time, the fallacies tend to become submerged.

Accordingly in this survey for the purposes of finding out which months are the most or least malarious, in other words to study the monthly incidence of malaria, the average monthly incidence over at least 10 years where figures have been available, were taken. In the comparative yearly figures which have been studied the error is probably negligible (*see* Appendix to Part III).

It is often stated that a certain proportion of a malaria-sickness rate is due to relapses of an infection perhaps derived elsewhere and must not be reckoned in considering the malariousness of a locality. The experience of Europeans going home on leave shows that this must be true to some extent but there is evidence that this factor is but a small one in the malarial sickness of Assam. If jungly coolies who are said to be 'particularly immune' to malaria come to healthy non-malarious gardens, they are not instrumental in spoiling the reputation of such places for non-malariousness in spite of their systems being sodden with the infection, in other words the sickness rate due to relapses is economically negligible. Moreover, if the cold weather is conducive to relapses, one should not find that the coldest months are the least malarious as they are.

An analysis has been made of the spleen-index as apposed to the putative health of certain estate cooly lines, with the result given in Appendix IC.

It will be seen that in lines with a reputation of being 'non-malarious' or 'quite healthy' 43 per cent in the Surma Valley and about 70 per cent in the Assam Valley have spleen-index of 21 to 40 per cent.

Those with a reputation of being only 'slightly malarious' had in the Surma Valley a spleen-index of 61 to 80 per cent in about 4 per cent, and a spleen-index of 41 to 60 per cent in about 28 per cent; while in the Assam Valley they had a spleen-index of 61 to 80 per cent in about 9 per cent, and a spleen-index of 41 to 60 per cent in about 38 per cent.

The converse is also true, some estates with a reputation of being very malarious have a comparatively low spleen-index.

Another factor of some importance in introducing errors into the malaria-sickness rate lies in the diagnosis. Malaria with its 'protean manifestations' is often a difficult disease to diagnose. Even the parasite in the blood may only be a coincident circumstance. Other diseases may be confused with it, e.g., kala-azar, paratyphoid, phthisis, influenza and nasal catarrh, septicæmias, *B. coli* infections. It seems probable that certain infections or illnesses cause the appearance in the blood of the malaria parasites which would otherwise lie dormant and that the symptomatology is then one of both the exciting condition and of malaria, or if as sometimes occurs the malarial element is not prominent, then of the former alone.

The malaria-sickness incidence for the whole of the Assam Government Hospitals and Dispensaries in 1921 has been kindly given me by Lt.-Col. Innes, Offg. Inspector-General of Civil Hospitals, as 3,32,387.

(*h*) *The mortality index.*

Deaths among coolies on separate estates are fortunately not sufficiently numerous to make this index of much value. In view of what was said above on the difficulty in diagnosis of malaria, figures based on the stated cause of death must be unreliable.

FACTORS IN THE ENVIRONMENT OF HUMAN HABITATIONS SELECTED FOR STUDY.

(*a*) *The Conformation of the Land.*

The correlation of malarial endemicity with the lie of the land was part of our study.

The old idea, no doubt originally derived from Rome, was that swamps were dangerous and living on a *tila* safe. On the other hand the conduct of certain Oriental races has evolved along different lines. It therefore seemed advisable in a country where there was no prevailing sentiment in the matter to enquire into the matter *de novo*.

The problem was difficult. In Assam there were rivers and streams and streamlets, paddy-fields, tea-drains, wells (not tube), tanks and their overflows, sullage water from factories, bhils and jheels, rivers dead and dying and active, high-banks, low-banks, hills and tilas, kals and hulas, buffalo-wallows, borrow-pits and ponds. The problem was to ascribe to each of such elements in the scenery its proper proportion of blame in the ill-health of the gardens. This side of the subject is dealt with in Part IV.

I derived much help in it from various text-books of physiography, Ferguson's 'Recent Changes in the Delta of the Ganges,' 1863, the maps of the Survey of India, sketches of the Estates kindly given me by the Managers, and finally from Officers of the Geological Survey of India, in Calcutta, particularly Dr. Coggin Brown. To the gentlemen who have helped me I am much obliged.

The physiography of the Assam tea gardens and the surrounding terrains is expressed in the following formula :—Estates established within the two great valleys of the Brahmaputra and the Barak (Surma) between which the Shillong spur or the Khasia-Jainti hills composed of rocks characteristic of Peninsula India intervene. These valleys are in the words of Mr. Coggin Brown 'gigantic *khadar* or straths, the greater portion being liable to flooding and consequently not in a habitable state. There are, however, higher tracts here and there, sometimes mere mounds, rising a little above the general level, and sometimes small plains which may be considered as representing the extensive *bhangar* of the Gangetic plains, while along the foot of the hills, there are gravel deposits usually of small extent.'

The *bhangar* of Assam as far as tea-lands are concerned is represented by the 'red-banks' of Tezpur, Nowgong, Lakhimpur and parts of the Surma Valley, and they form a very important part of these lands. Wherever tea can be grown, i.e., any land which can be drained deep enough, may be called *bhangar*.

The natural drainage system of these great valleys exhibits on a great scale all the phenomena of water dynamics and water physiography and is, of course, the most important element in the malarial epidemiology.

With but one or two exceptions the gardens we investigated lay in relation to the above described conditions and we have not included in our consideration of the subject anything dealing with the malaria of the Shillong hills. On the other hand, a few references to the essentially similar tracts of Bengal have been made, following Fry's Reports (1912 and 1914).

FACTORS ASSOCIATED WITH THE CONFORMATION OF THE LAND.

(a) *The mosquitoes.*

The idea was to associate certain known mosquito-carriers of malaria with its incidence, taking into consideration either the relative seasonal prevalence of the adults, or the numbers of their breeding-places and their distances away from the focus of the disease.

Of the approximately 40 accepted Indo-Malayan Anophelines,* 15 have been found in Assam (for list *see* below in Part III), and of these, because of what is known of their activities elsewhere (*see* Appendix IIID), 6 must be on their face-value suspect. These will be shortly considered here. They are :—

umbrosus
jeyporiensis
aconitus *
funestus * (= *minimus* + *listoni*)
culicifacies
maculatus

Concerning them and their activities I will cite the remarks which I made at the Jorhat meeting of the Assam Branch B. M. A., 1925.

umbrosus. This species was one of those responsible for so much trouble in Malaya before Watson showed how to deal with it. We now know it is very easy to tackle ; rough open earth drainage even through the jungle or in densely-shaded places, so that by merely cutting down the shade one becomes rid of it.

As it can be so harmful, the question arises as to the place it takes in the malaria problem in Assam.

I have caught it in several places in Assam jungles, for instance, in the swampy ground under jungle near to Lokra in the Balipara Frontier Tract (Fig. 2), and I have no reason to suppose it is not to be found in all similar situations.

But does it do any harm ? I agree with Watson that in gardens which are well-cleared, such as in the Doom Dooma district, the species is perhaps of no importance, nevertheless it is significant that of the estates along the Dibru or similar rivers, which he mentions as being so malarious, some are opposite the Dibru Forest Reserve. The evidence of the seasonal incidence of malaria in the gardens is also compatible with this being at least partly due to *umbrosus*, for, being a swamp breeder, it is probably much increased in numbers during the rains, when malaria is also more prevalent.

*** Part of the Assam Valley is classed by Zoologists as Malayan in its fauna.**

At Pasighat, which Lt.-Col. McCombie Young, I.M.S., surveyed, *umbrosus* was not incriminated, but I am inclined to think on reading his description of the terrain that *umbrosus* may have been partly to blame. It seems to me significant that great improvement in health followed cutting down a belt of jungle 300 yards from the lines.

Then one must note that at Lumding Junction, where all anophelines except some breeding in the bed of a river have been got rid of and the spleen-index reduced from about 80 to 30 per cent, a community of sweepers living near the jungle boundry still have a high malaria rate, and a splenic index of about 90, so Dr. Leitch tells me.*

As a final point I would cite the ill reputation for 'fever' that the Nambur Forest has for officers on tour.

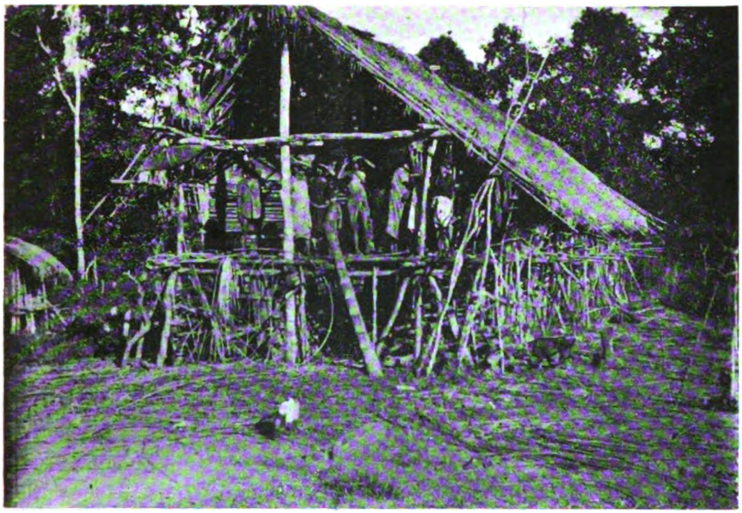


FIG. 2.—In the swampy ground under jungle near to Lokra.

In general I would give my opinion that any undrained jungle in relation to tea gardens should be considered dangerous until it has been proved not to be so.

A. maculatus. McCombie Young in his Pasighat Survey determined that *maculatus* was the cause of most, if not all, of the trouble. At Lumding, where it was found breeding in the sandy bed of the considerable river, the Horu Langpher (Fig. 2), it was not incriminated to the same extent by James, Young and Fry; and as subsequent anti-malarial measures seem to have eradicated the other species, one may conclude that *maculatus*, which still breeds in the bed of the river, is probably responsible for the spleen-index of about 30 per cent, which persists. I am indebted to Drs. Weldon and Leitch for my data.

---

* But Dr. Lees, I believe, dissents from this opinion.

Challam at Nalbari, in 1920, found no *maculatus*; in 1921, during October and November, a few; and in 1922, after April, some more. He concluded, nevertheless, that they were not the source of much malaria, in which view McCombie Young concurred. On the other hand, *maculatus* was one of the species which Watson considered to be a dangerous factor in the Doom Dooma gardens. He found it abundantly in January.

In general, McCombie Young (1924) states *maculatus* to be a most potent vector in certain parts of the plains of Assam.

I did not find *maculatus* myself very often, only 17 times out of a total of 2,774 catches of all species, and of those most were found in hill-side streams where one would expect the species; however, the paucity of my catch is not surprising when one considers the general lack of suitable breeding places for it in most tea gardens.

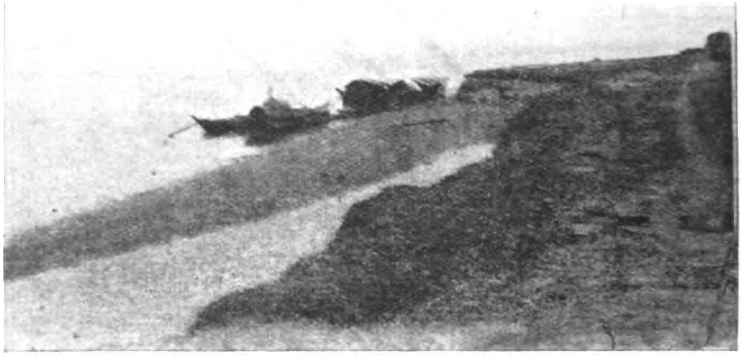
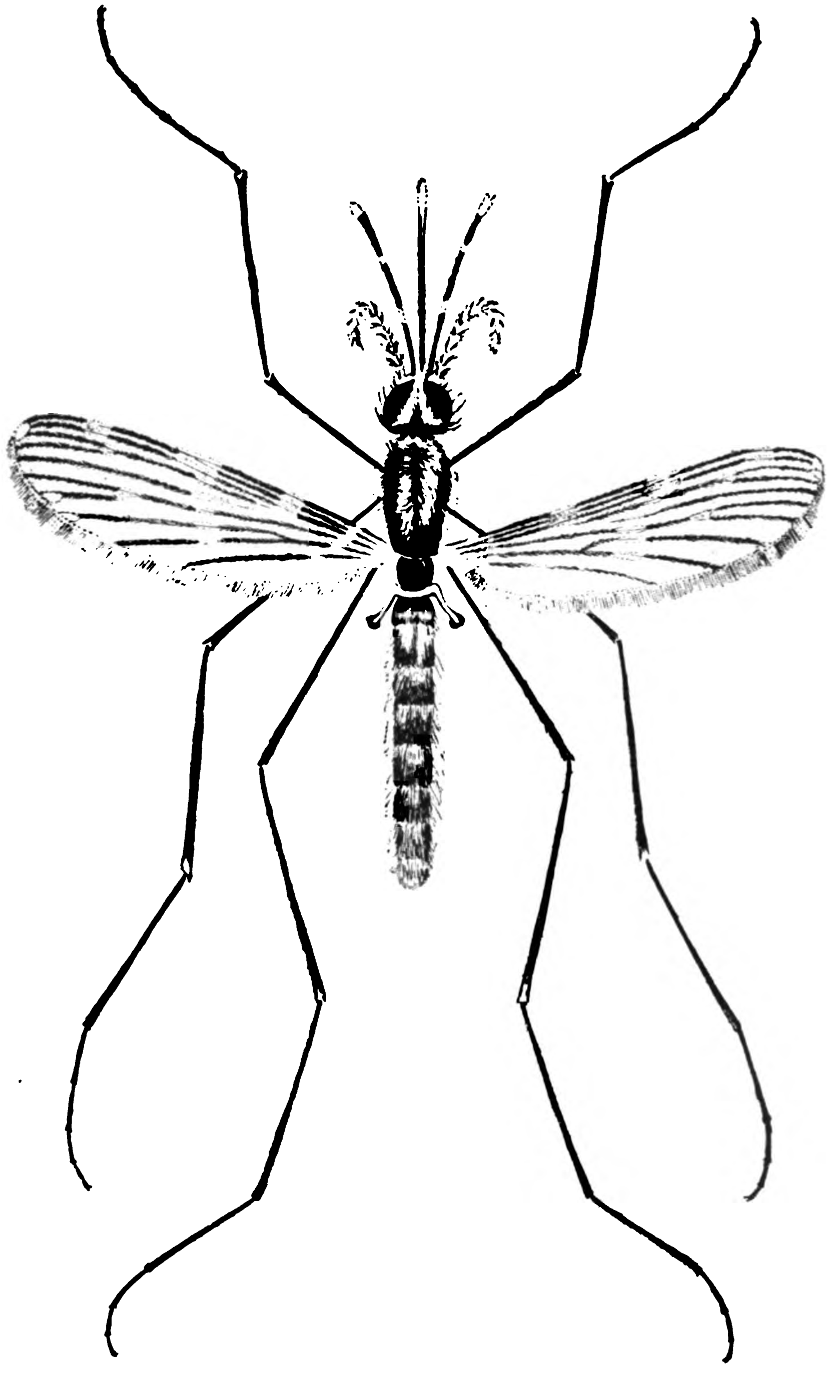


FIG. 3.—Breeding in pools in the beds of big rivers like the Brahmaputra.

A. maculatus is essentially a stream breeder (Fig. 3) or a drain breeder, so that where drains hold spring water they may be dangerous; it is not a swamp breeder. Watson says, '*A. maculatus* lives in the purest water, so it is found in hill streams, springs at the foot of the hills and banks, and in bhils. It is never found out in large flat *pathars* (rice-fields) away from hills, but in narrow valleys in the hills; it may be found in the small pottahs or areas which are irrigated by clean water. It is highly sensitive to pollution, or its food is, for even daily washing of clothes at the head of a small valley will drive the insect from the main stream.'

With regard to Watson's conclusion that the species must be regarded as one of the chief causes of malaria in the Doom Dooma gardens, it must be remembered that he found it in the months of January to March, and as this is not the malaria



A. culicifacies ♀.
Note the two pale spots on the wing fringe.

season its connection with *maculatus* was not thereby proved. No observer has yet reported it during the malaria season.

Watson says, and I agree, that the species is to be found breeding in pools in the beds of big rivers like the Brahmaputra (Fig. 3), but that 'big rivers as a rule are not associated with malaria,' which proves that the mere fact of finding *maculatus* in any place is not evidence of any connection with malaria there.

The species having such a liking for seepage water is an additional argument against it being very prevalent in Assam or causing much trouble, for the land is generally low-lying and there is not much seepage; what there is will be found where the land is more hilly as in Cachar (Fig. 17). In such places, in my opinion, it is more likely that *maculatus* is doing harm, although I found no direct evidence of it.

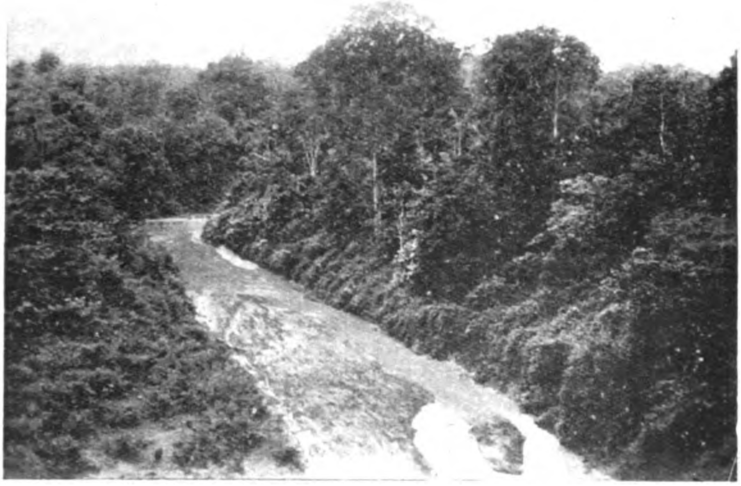


FIG. 4.—Found in pools in the bed of the big river.

A. culicifacies. This species has been reported in the Nalbari and in the Lumding Survey. At Nalbari it was found in June, breeding scantily in rain pools in paddy-fields, but not much importance was ascribed to it. At Lumding it was found by Weldon in large numbers in a tank as well as in pools in the bed of the big river (Fig. 4) and much of the malaria was put down to it. Unfortunately the season during which it was found breeding was not mentioned.

Watson caught none of the species but in his conclusion thinks that perhaps it is of some importance.

Likewise, during my three months' preliminary survey of the tea gardens in 1923, I found none. However, there is no inherent improbability that *culicifacies* is a factor in tea-garden malaria. Watson and I caught none because we tried at the wrong season (October to March). The species is a pool breeder like *umbrosus*, so it should be looked for at the advent of the rains when the malaria season is starting. It is a clean-water breeder; seepage or fresh-water pools in the beds of streams or drains in the uplands or where subsoil drainage is good are its natural habitat. It would not be found on low-lying lands like the *pathar* level, hence a place like Dibrugarh is not malarious. Drainage eradicates it as it cannot breed in running water.

A. jeyporiensis. This species, in the light of our present knowledge, need not be seriously considered.

I only found it on one occasion in South Sylhet; Dr. Meek has since sent (from Cachar) a number of specimens caught in August, but as no one during the previous surveys has reported it, it cannot be of any great epidemiological importance.

A. funestus (*minimus*) and *A. aconitus.* I will deal with these two species together as they are closely related. It is not usual to find them on the *pathar* level; their habitat lies in streamlets of running water edged with grass (Figs. 5 to 7). The larvæ have the thorax particularly well developed, which enables one to distinguish them from most other anophelines with the naked eye. Watson says they are found in the clearest water, e.g., 'in the bhils.'

McCombie Young in the Lumding Survey and Challam and McCombie Young at Nalbari decided that in these places *funestus* was the chief carrier, while Watson in Doom Dooma concluded that it was of the greatest danger to the tea gardens; he found it in abundance and of adult anophelines caught in houses, 45 per cent were of this species.

I found 136 breeding-places out of 1,730 during September to November 1923, *umbrosus* being the next among the malaria-carriers with 29. The species was not found in comparatively non-malarious localities.

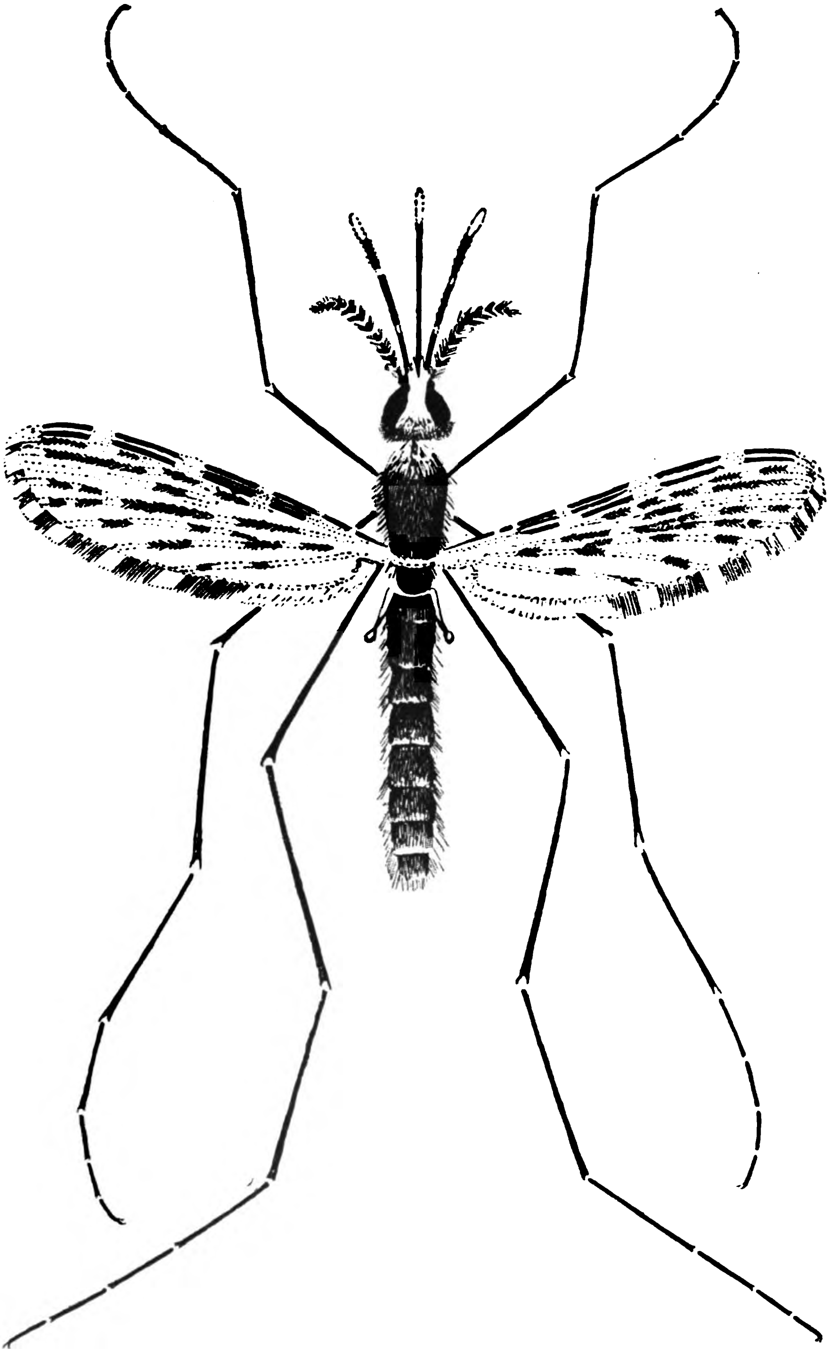
It is generally agreed then that this is an enemy of the first importance which is equivalent to saying that the streams and irrigating channels of the rice-fields are culpable (Fig. 12).

Conclusions regarding the relative importance of the species.

Of the known malaria-carriers which occur in Assam, I have concluded then that *funestus* and *aconitus*, represented in the paddy-fields, are very dangerous; that probably during the rains *culicifacies* breeding in pools of pure water is also of danger; that *maculatus* breeding in spring water cannot on present evidence be incriminated with doing much harm; that *umbrosus* lurks in all undrained jungle which should be viewed with great suspicion; and that *jeyporiensis* takes no important part in the causation of the disease.

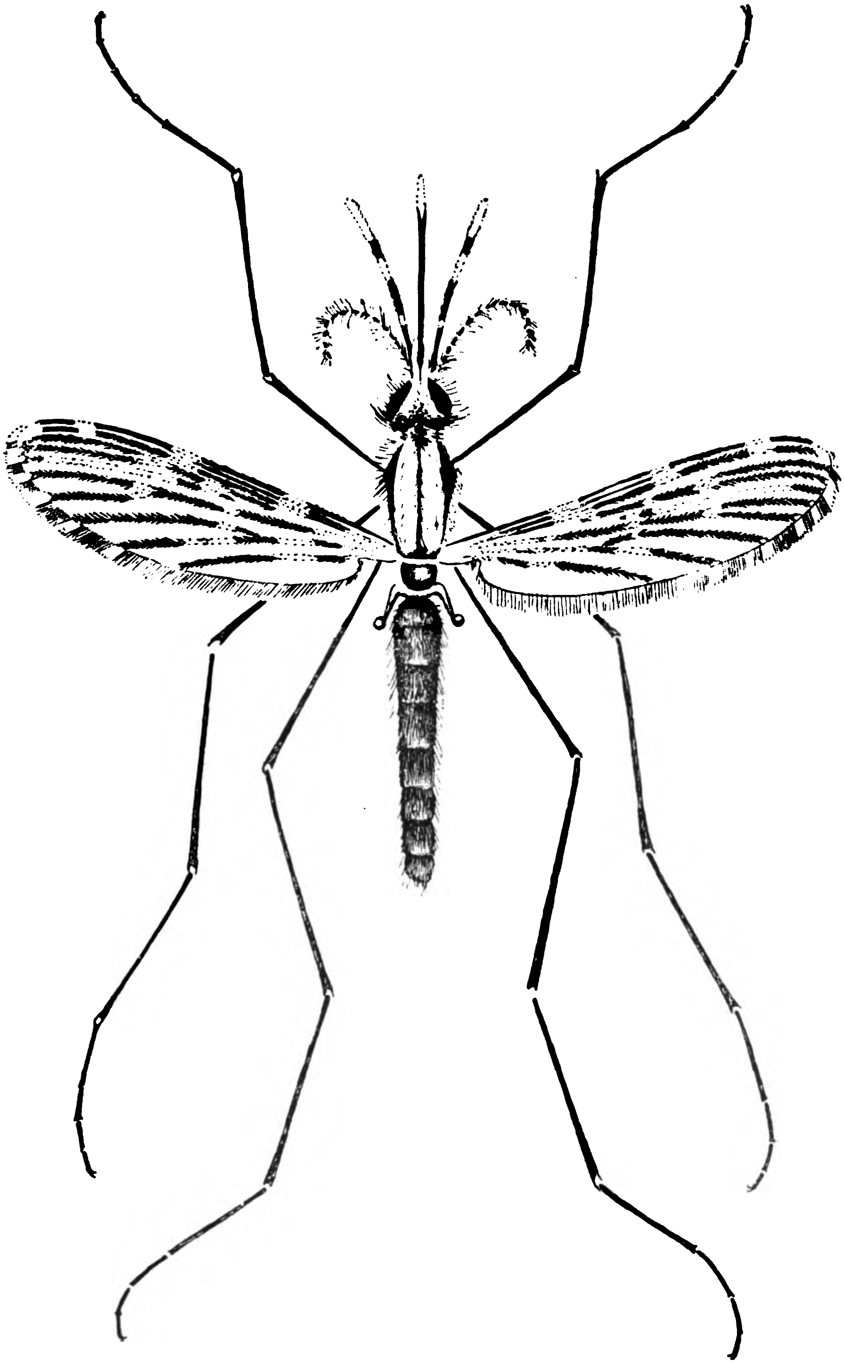
In connection with the laboratory technique involved in the mosquito survey, it must be noted that by far the largest proportion of the identifications made

PLATE II.



A. jeyporiensis.
Note the small spots on the hind legs and the two small spots on the inner third of the wing costa.

PLATE III.



A. funestus ♀.

Note the lack of a pale spot on the wing fringe opposite the sixth vein.

were from the larvæ, and at this stage diagnosis cannot be always made between two or more species, especially during the early instars; e.g., *willmori* and *maculatus* and *karwari*: or diagnosis may depend on very fine points not always easy to make out, thus in *jamesi* and *fuliginosus*, or in *culicifacies* and *rossi*. On the other hand while one may be able to diagnose the larvæ of two species, e.g., *vagus* and *rossi*, one may not be able to do so in the adult state, e.g., *vagus* and *rossi* males. One may therefore lump the findings of certain species together, rather than submit them under specific headings. For example, instead of this list:—

vagus	F.	10
rossi	F.	11
vagus or *rossi*	M.	24
vagus larva		10
rossi larva		3
culicifacies	F.	1
culicifacies	M.	4

One might say *vagus, rossi* or *culicifacies* 63, and then specify the numbers of those which had been certainly diagnosed.

Finer diagnosis can be effected by hatching out the adult from the larva and examining both the larval skin and the adult, but this adds to the labour enormously.

The following is the list of mosquitoes caught as larvæ during the Preliminary (1923) Survey:—

Species.	Larvæ.	Adult. F.	Adult. M.	TOTAL.
jamesi or *fuliginosus*	339	..	..	387
jamesi	..	..	1	
fuliginosus	..	20	27	
barbirostris	247	41	46	334
sinensis	273	22	30	325
vagus	218	13	42	273
sinensis or *barbirostris*	135	..	..	135
funestus	78	5	19	102
tesselatus or *kochi*	62	..	..	..
kochi	..	8	12	89
tesselatus	..	..	2	
leucosphyrus	2	2	1	
aconitus	33	1	..	34
umbrosus	25	2	2	29
maculatus or *karwari*	13	..	..	17
maculatus	2	..	..	
karwari	..	2	..	
aitkeni	4	..	..	4
undiagnosable	..	..	..	34
undiagnosed	..	..	..	11
				1,774

The following were the specific breeding-places found during the 1924 survey:—

Species.		Number unequivocally diagnosed.
hyrcanus (*sinensis*) with *barbirostris*	1,133	792 215
vagus with *culicifacies* and *rossi*	452	
fuliginosus with *jamesi*	366	2
aconitus with *funestus* (—minimus)	51	
kochi with *tesselatus*	46	
maculatus with *willmori* * and *karwari*	1	
TOTAL	2,049	

In the matter of the distance of the breeding-places of anophelines from sites of habitations we aimed at mapping out an area extending to about a half mile around them. Watson (1911) had shown that breeding-places of a strong flier like *umbrosus* were economically of but little importance over a quarter mile away.

No systematic search in this survey was made for adult anophelines but a few were caught as occasion arose. The species of mosquitoes that gain access to human habitations in various localities is, however, an important point. There are many checks to the mosquito between its breeding-place and its human prey.

(*b*) *The seasons.*

An attempt was made to correlate health (or lack of it) with the seasons and associated factors (*see* Part III).

The data in this study were obtained from the Meteorological Department, which kindly supplied all those applicable to the localities worked in.

The average rainfall for all the stations of Assam for 10 years was 112·26 inches. The average daily maximum temperature 84·8 per cent and the average minimum 66·6 per cent.

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* Has never been certainly found in Assam.

Conditions associated with the seasons.

The only data collected under this head were the anopheline species and the relative number of specific anopheline breeding-places. With regard to recording the latter the human equation must be allowed for. During the rains or very hot weather it is obvious that larva-collecting will not be so ardent as during fine cold weather.

It would have been interesting and important to have caught systematically the adult anophelines in human habitations and dissected them to determine their relative rate of infectivity during the seasons. This work, however, would have absorbed a great deal more labour than we had available, and while the results might have been very valuable in assessing the relative importance of the mosquito species, it was thought better to proceed on the assumption of 'Once a carrier always a carrier' and to devote the available time to the other sides of the survey.

Means and Methods.

The data for our survey was collected in two parts, first during a preliminary reconnaissance by myself from September to December in 1923 and then, specially from the seasonal point of view over the whole of 1924, this detailed survey being in the field in the charge of Dr. K. L. Chowdhury.

Staff and Organisation.

In the Preliminary Survey it was the intention to train on the staff in the duties necessary for the collection and record of the data during the year's detailed work that was to follow. Dr. K. L. Chowdhury, M.B., D.P.H., was appointed as Chief Assistant, but it was found difficult to get men of sufficient intelligence to take much interest in the district field-work, and constant changes had to be made with the result that at the end of the first lap the only survivor among those who had been recruited and trained declared his inability to stand the hardships any longer. Nevertheless, with the assistance of Dr. Chowdhury, the temporary help of the above-mentioned sub-assistant surgeons, a laboratory assistant, a clerk, and a batch of Paharias for the field-work, I managed to collect and record a good volume of data, which was the subject of the Preliminary Report already referred to.

In the detailed survey which followed, Dr. Chowdhury started afresh to train on as rapidly as possible a batch of new sub-assistants. It was arranged that there should be three of these, one for Lakhimpur and Sibsagar Districts, one for Darrang and Nowgong, and one for the Surma Valley. However, when the gentleman in Darrang strongly objected to the Mangaldai bears and found it more convenient to travel to his work on the gardens by train from the Civil Station at Tezpur, I gave him and the local scheme up in disgust and confined the work to the other districts.

A monthly type-written formula for collection of the data was laid down and issued to the supervising officer and each field-assistant; but the output, although large (as will be seen in the body of the report), was but a fraction of that ordered, and I have come to the inexorable conclusion that the type of man

whom we recruited, however capable medical man he may be, is of no use for
sort of work, and another type will have to be found.

At the conclusion of the field-work Dr. K. L. Chowdhury and subseque
Dr. J. B. Mukherji were engaged to assist me in compiling tables from w
this report issues, and they have done this very efficiently.

Acknowledgments.

I owe a great deal to Lt.-Col. Megaw for his sympathetic administra
of the scheme, also the officers of the Indian Tea Association, in Calcutta
in Assam, and the Executive Staffs of the gardens, who gave all pos
assistance.

I also feel that my gratitude cannot be adequately expressed to the Me
Officers of the Estates for their ready acceptance of the spirit of the investiga
and for the great help given to me and my staff in obtaining the data which
required; my own part really has only been that of collecting and forwarding a
for their parcels of wisdom. Indeed, believing it would be of great interest
value, I have persuaded Drs. Dodds-Price, C. E. P. Forsyth, D. P. Williams, H
Smith, and Murphy, to contribute in Part II of this report their several p
of view as representative of the opinion in the more important districts of
Estates Doctors. I am specially indebted to these colleagues for the tro
they have taken in the matter.

And not only to the above gentlemen must I return my thanks but als
the Assam Government and to many of its officers, both civil and medical.

In the sequel the obligations due to all and each will be more particu
acknowledged.* Apologies are due to those whose names have inadvertently
wrongly spelt.

[In the original report the three appendices mentioned in the Table of Cont
were here attached, but are now filed for reference in the office of the I. T
Calcutta.]

---

* The names of the doctors to whom I am so specially indebted for giving
their valuable time in order to show me their estates are as follows:—Drs. Fal
C. E. P. Forsyth, J. Dodds-Price, G. E. C. Ramsay, D. Gibson, Hugh Smith,
R. A. Murphy.

PART II.

CONTRIBUTIONS BY ESTATE MEDICAL OFFICERS.

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Chapter I.

NOTES ON MALARIA ON THE TEA ESTATES OF THE NOWGONG DISTRICT, ASSAM.

By

J. DODDS-PRICE, M.R.C.S., L.R.C.P.,

Medical Officer to the Salana Tea Co., Ltd.

It must be clearly understood at the outset that these few notes on malaria are not the outcome of a special study of the disease. A year after I came to the district, kala-azar had obtained a very firm footing on most of the tea estates under my care, and from then on, for several years, my time was almost solely occupied in fighting this disease. It was recognised that kala-azar was a distinct condition, and this opinion was firmly maintained in spite of adverse criticism from various quarters. It had appeared to me to be a soil infection. By taking the splenic index on several of the estates under my care after kala-azar had been stamped out of them, and by examining blood slides from the children with enlarged spleens, malaria parasites were found in about 80 per cent of the cases. On estates where kala-azar was still much in evidence, the malarial figures were practically the same. These figures were confirmed by Captain S. P. James, in 1904, and it is my considered opinion that malaria is no more prevalent on estates where kala-azar has raged or is still present, than on estates free from this disease.

Certain epidemics do, I think, affect the malaria incidence, and it was noted on two estates severely affected by influenza during the hot dry months that during the following rainy season the admission rate from malaria was much higher than in previous years.

The most malarious period on tea estates in the Nowgong District is from the end of July to the end of October. It varies in intensity from year to year and is usually more severe if the rains are excessive than with a scanty rainfall. In this connection it may be pointed out that in August 1923 some of the estates had less than a quarter of their normal rainfall, barely five inches falling on the estates in the centre of the district. On these estates the incidence of malaria did not attain its full force until September which was a wet month and admissions under malaria rapidly went up.

Estates which suffer severely from malaria during the rainy season show a much larger proportion of malaria cases during the cold weather months than on estates where malaria is less severe and where admissions during the rainy season are fewer.

These more malarious estates are intersected by deep 'hoolahs' which it is not possible to cultivate and which are overgrown with jungle and full of water* during the most trying period of the year. Estates surrounded by open country with large stretches of paddy cultivation in the immediate vicinity usually show a much smaller admission rate from malaria, and the general health of the coolies is much better.

General lack of conservation adds to the malarial incidence, and it has often been pointed out to estates managers that it is quite futile to go in for an elaborate cleaning-out of drains during the cold season unless these drains are kept open and clean during the time of year they are most required. Twenty years ago there was a large proportion of 'jungly' coolies on most estates in this district, and yet the health of the coolies was not so good as it is at the present time, in spite of the fact that the class of coolie is now inferior from a working point of view and kala-azar is to be found in most estates. The improved health is due to the better conditions under which the coolie lives on all the estates, for owing to the severe epidemic of kala-azar that swept through the district the old lines were completely destroyed and fresh lines provided on healthier sites as far as possible, and more attention paid to drainage and sanitation.

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* Stagnant or otherwise. In some of these 'hoolahs' in the vicinity of a river (stream) the water rises and falls with the stream water.

We have never gone in for elaborate coolie houses, and the type in my opinion matters but little, provided the houses are well raised and the floors in consequence always dry. Galvanized iron roofs have largely superseded thatched roofs and angle iron for posts and beams is economical in the long run and does not harbour 'live-stock'. But all things being equal, the estate that has the largest labour force *per* acre is the estate that keeps the healthiest. If the coolies are able, during the busiest months, which are also the most malarious, to take an occasional day off in addition to the usual leave day, they keep better health than those on an estate where labour is scarce and work has to be carried on at high pressure. Estates surrounded by paddy land which the management is able to hand over to the coolies for purposes of cultivation have usually the lowest sick rates, are the most contented, and show the lowest malarial rate, and the smallest sick list.

Cold wet weather sends up the malaria rate. Coolies can stand repeated soakings while at work, provided the temperature remains high, but a drop in temperature due to continuous heavy rain always increases the admissions from malaria.

It is many years now since newly imported coolies came up in such numbers to any estate in Nowgong to enable one to express an opinion as to the effect on the malarial incidence. In 1918, coolies from famine-stricken districts trekked up to the tea estates of Assam literally in thousands, but any figures one might have accumulated on the malarial incidence would have been completely stultified by the fact that the pandemic of influenza was raging at the time. Most estates were suffering badly, on many over 70 per cent of the labour force was attacked, and the arrival at frequent intervals of half-starved immigrants did not help matters.

Estates that suffer considerably from malaria in addition to showing a higher death-rate have a much smaller birth-rate, not that the women do not become pregnant but because of a higher proportion of abortions and miscarriages, which are only *exceptionally* due to the use of abortifacients.

When coolies came up to Assam under a Penal Act, one of the laws laid down by Government was to the effect that pregnant women were to be given one month's leave before child-birth and one month after the child was born. This did not meet with my approval at all; a healthy pregnant coolie woman requires practically no leave before child-birth. If she is not fit, let her have sick leave, and on big estates she is lost sight of and the next thing one hears is that she has had her child, but it is dead. After child-birth I insist upon at least 4 months' leave, not only for the woman's sake but for the sake of her infant and on the majority of my estates the women on confinement-leave appear before me once a week and the infants are inspected. Bonuses are also given at the end of a few months to women whose infants have been well cared for. The following comparative figures may prove of interest. They are taken from four estates, all with a population of between 1,500 and 1,600 souls. Two of the estates are unhealthy from a malarial point of view, intersected with deep 'hoolahs' and in the vicinity of much forest and jungle, and two decidedly healthier in open country with much paddy land under cultivation.

Unhealthy Estates.

During the years 1921, 1922 and 1923.

		1921.	1922.	1923.
No. I.	There were 137 deaths from all causes	43	34	60
	" " 95 births	42	24	29
No. II.	There were 76 deaths from all causes	27	21	28
	" " 69 births	24	26	19
	Healthy Estates.			
No. I.	There were 40 deaths from all causes	11	11	18
	" " 112 births	43	35	34
No. II.	There were 63 deaths from all causes	18	21	24
	" " 118 births	30	41	47

It will be observed that on unhealthy estates the deaths far out-number the births; on healthy estates the births on the other hand greatly exceed the deaths.

The question of quinine prophylaxis in combating malaria is, I believe, a very vexed one. I tried it on two large estates which had very big admissions from malaria and the results appeared to me to be decidedly encouraging. To be successful it must be done thoroughly and with the co-operation of the management. It cannot be left to the Doctor Babu. The plan adopted was to administer a daily dose of 5 grains to the entire labour force from May to October 15th. Either the manager or an assistant was present at the morning muster and no shirking was permitted.

In the Duars black-water fever among Europeans was so bad a few years ago that a commission was appointed to enquire into the question. Among the findings it was advised that a daily dose of 5 grains quinine hydrochloride be taken every day from January 1st to December 31st. I paid a visit later to Dr. William Brown who had then practised in the Duars for over 20 years and he was convinced that this daily dose of quinine had helped to check attacks of black-water fever in a most remarkable degree. No repeated attacks of malaria, no black-water fever.

When officiating as Civil Surgeon of the Nowgong District, 1916—1920, quinine prophylaxis was carried out in the Nowgong Jail from May 1st to October 31st. In the adjoining Police Lines no such measures were adopted and the result was most striking. Admissions to the Police Hospital from malaria were of frequent occurrence. In the Jail admissions were very few.

Recently, for 2 years, quinine prophylaxis was stopped in the Nowgong Jail and the malarial admissions immediately went up. Prophylactic treatment was consequently started again with very favourable results. Five-grain doses in solution were given every morning.

In the Nowgong District the coolies from Chota-Nagpur keep the best health, i.e., Santals, Oraons, and Mundas; Bhumij, Kumars and Lohars also do well. Coolies from the Lower Central Provinces, Bilaspore, usually keep very fair health, but the castes should be carefully selected—Goala (Ahir), Chamars and the like being unsuitable. United Provinces people are most undesirable and should never be recruited. It is also most unwise to import people to labour on the tea estates of Assam unless rice is their stable article of diet.

Chapter II.

NOTE ON MALARIA IN TEZPUR.

By

CHARLES E. P. FORSYTH, M.D., D.P.H.

Tezpur is justly accounted one of the most malarious districts in the Ass Valley.

The majority of the estates, roughly about 80 per cent of the total acre of tea, are situated on the red-bank, a formation characterised by a medium-hea soil cut up in a remarkable way by 'hullahs' or shallow ravines, and many the gardens abut on forest continuous with the immense, wide-spreading grow running up into the foot-hills of the Himalayas. On certain portions of red-bank off-shoots from the main forest or isolated areas of tree-jun survive of varying density and extent thrust into the tea or form boundaries of estates. The few gardens on 'dhan-khet' level enjoy compa tively good health, and it may in fact be clearly affirmed that the nea the forest the unhealthier the estate. The area as a whole, however, be correctly described with regard to malaria as one of hyperendemicity. spleen-rate in the children up to about 5 years of age is round 90 per ce in children of working age to 15 years it is 30 per cent, and in adults index ranges from 6 to 10.

In about 70 per cent of the cases of malaria that come up for treatment infection is sub-tertian. Quartan fever occurs but rarely, being found fr time to time in one or two definite localities. Mixed infections are no do common. Black-water fever is endemic, never, however, affecting the co population, but occurring amongst the Babu class, Chinamen and Europeans, severity of the attack being definitely in the order named, the European suffer most.

As may be gathered from the spleen-index, the incidence of mal amongst garden-coolies is exceedingly high, and the direct economic enormous.

The following table shows figures over a number of years, taken from se gardens, of cases of malaria and the attack rate per hundred of the working pop tion. Malaria cases amongst adults and children are grouped together, so the lab loss is not actually so much as shown, but when a child is sick the mother or fat has usually to be away from work to attend on it, and the excess in the figures is not, therefore, very great.

TABLE.

Year.	Malaria Cases.	Working Population.	Attack Rate.
			Per cent.
1915	16,587	6,632	250
1916	19,135	7,165	268
1917	17,596	7,291	241
1918	10,650	6,848	155
1919	9,838	8,081	121
1920	6,951	8,622	80
1921	5,184	8,071	63
1922	5,621	6,908	81

Attempts have been made to combat this heavy loss, and the employment of quinine as a prophylactic was for many years the method relied on. This proved an utter failure and only added to the wastage. The cost of the drug was high, it was mainly administered in the expensive shape of sugar-coated tablets and the effect on the volume of disease was non-apparent. There can be little doubt of the value of prophylactic quinine against malaria when the question is fairly examined, but for the attainment of success details of its administration have to be carefully gone into, and the haphazard giving of the drug to labour forces exposed to continuous intensive infection can never afford a hope of benefit. Money and energy were diverted from what might have been the obvious line of attack, viz., that against the mosquito and the ground lost has been difficult to regain. A serious handicap, however, in the taking of this course was the prevailing ignorance of the actual carriers of the infection. No mosquito-survey had been attempted, no exact knowledge was available regarding the species of anopheline mainly concerned in the crime, and anti-mosquito work at the best tends to be expensive, although when accurately directed the cost is recoverable to an incalculable degree. In 1919, however, a certain definite advance was made after some experimental work carried out in the previous year, and the extravagant and valueless methods connected with the indiscriminate use of prophylactic quinine were put an end to. There followed not only a decided gain financially but a clear gain in the incidence of malaria amongst the labour forces, for it was replaced by systematic treatment with the drug of each case of the disease as it arose, the sufferer being followed up and subjected to a course of quinine sufficient to fortify him at least against early relapse. This produced a marked fall in the case-rate.

In addition to what may appear to be the rational employment of quinine, efforts have been made to free the gardens from jungle on the red-bank by the clearing of 'hullahs' and their cultivation with rice for which these areas are eminently fitted, and in these ways notable success has been achieved on certain estates.

The doubt can be urged whether a change of this sort, the clearing of jungle may mean merely the substitution of one malaria carrier for another. It may be so but experience has gone strongly to show that the forest or jungle-breeding anopheline is the danger as may be seen from the marked improvement of health demonstrable in these estates where these methods of 'hullah' cultivation have been carried out. In the consideration of the question, however, it cannot be ignored that besides the lessening of the breeding-places of dangerous anophelines there is the advantage of a fuller source of food supply to the worker, and this certainly can be no small matter in building up a resistance to malaria and disease in general.

An important question closely bound up with the prevalence of malaria is the acclimatisation of new labour, and the impression obtained as observation and experience increase is that the influence of the disease is far-reaching if not fundamental. Types of people—third and fourth class labour—who live and work well on the 'dhan-khet' estates would rapidly sicken and disappear on the gardens of the red-bank, and one of the main factors of the phenomenon may well be malaria, accepting the more potent influence for evil of the forest-breeding anopheline. The malarial survey will throw light on this so far as this district of Tezpur is concerned, a pressing problem in the present difficult conditions affecting the recruitment of coolie labour.

Certain points call perhaps for special notice. The seasonal incidence, for example, of malaria in this district is very definite. In records kept over a series of years the curve shows a very steep ascent to the period July-August from March-April, and a correspondingly sharp fall. In the cold weather the malaria admission rate is rather less than half that in the rains, the proportion calculated over a period of eight years being approximately as 37 to 79. The definite seasonal variations can well be accounted for by the distribution of rainfall, the year to year variation being extremely slight, the curve following closely the months indicated.

There is no appreciable connection between the price of food-stuffs, or variation in wages, and the incidence of malaria. It is reasonable to suppose, however, that where the general resistance is lowered by under-feeding or privation, the susceptibility to disease is increased.

Epidemics of other diseases do not materially affect the incidence of malaria, except possibly, as apparent in the influenza outbreak, when a certain amount of confusion in diagnosis occurs.

The relationship of the incidence to paddy growing and the clearing of jungle has been touched upon, and as to coolie lines there can be little doubt of the benefit of good housing conditions. Barrack lines of dark back-to-back dwelling affording amongst other evils ideal lurking places for mosquitoes and the situation of houses close to jungle-grown ravines or 'hullahs' cannot fail to be harmful. The keeping of domestic animals, cows and buffaloes in katcha structures beside and between line houses adds to congestion, but the provision of separate cow-sheds for coolie lines has only in a few cases been found practicable. The pukkha house with corrugated iron roof, fitted for one or two families and properly spaced is the most hygienic type of dwelling, but the iron roofs render the houses subject to extremes of temperature and a subsidiary roof or 'chang' is as a rule constructed by the inhabitant himself to modify the excessive heat in summer and the cold in winter. Thus, when the usual katcha lean-to is added for the accommodation of cattle and a 'bari' planted out, there comes to be little difference between this class of house, and that covered with thatch and katcha throughout, as harbouring grounds for mosquitoes.

With regard to the effect of malaria on infantile mortality and the general health of the survivors there is, of course, no doubt of the acquirement of an immunity to the infection apparent in the marked decrease in the spleen-index with age. Infant mortality is appallingly high, but the causes are to a degree complex and involved, and it is impossible to state how much of the evil can be correctly ascribed to malaria. Coming to the survivors, however, in the average coolie lines the exuberant health, happiness and general well-being of the child population from about 18 months or two years upwards, are there for all to see, the possession of a large spleen or not appearing to make little or no difference to the individual. At the period 10 to 15 years the children begin to fall off again, when other factors become operative, although then the spleen-index which they present may be appreciably less.

An influx of new coolies to an estate means invariably a high malaria-rate, and as I have endeavoured to point out, the acquirement of immunity to malaria amongst these people is a most important factor in their acclimatisation. Amongst different races of coolies, malaria rages indiscriminately, the main point being the newness of the people to the country. The more robust classes withstand the infection, however, as evidenced in this district by the comparative readiness of acclimatisation of aboriginals like Mundas, Oraons, and others as compared with people, for example, from the East Coast.

Chapter III.

MALARIA IN THE LUSKERPORE DISTRICT, SOUTH SYLHET.

By

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This district is situated at the extreme south of the Surma Valley, an climate and soil differ considerably from those in other parts of the province. cold weather is shorter, the district is liable to experience severe droughts befor rains proper break, and very little rain falls outside the period of the mons The hot, dry winds which blow in March, April and May do not obtain fu north ; a shade temperature of 100 and over is not uncommon, and during t months the scarcity of water becomes serious, wells of even 35 to 40 feet run dry. The monsoon is usually ushered in by one or two very heavy falls of which may measure from 8 to 13 inches in twenty-four hours, after which of 2 to 4 inches occur. The monsoon blows harder than in most districts, t are few breaks and little sunshine. The yearly rainfall is from 80 to 100 in The soil constituents are lighter than are usually found, and its sandy na is very apparent.

The configuration of the region is that of a valley lying between two ranges of hills. The hills themselves appear to be unsuitable for tea and are planted, the estates being confined to high ground abutting on the hills. land is made up of 'tilas' (small hills) or plateaux, with intervening gullies ravines containing bhil, or if not too low-lying, rice land.

Although the malarial incidence is not so heavy as is found in some o severely affected districts, it is above the average, the spleen-index being l and there are few Europeans living in this district during the malarial s who escape infection. In this connection, considering the prevalence of ma on the tea estates of Assam, often of the sub-tertian type, the low death invaliding rates of the European residents, due to malaria, are rather remark I attribute this to the fact that the planter is continually under the observa of his doctor, and medical attendance being provided, he does not hesitate t advice for trivial complaints or slight fever. Moreover, the planter is abov average as a patient, is not influenced by cranks, and, as a rule, willingly ac treatment prescribed.

In this district, quartan malaria occurs occasionally, benign tertian is most frequent, but probably 30 to 40 per cent of infections are sub-tertian. Cases of black-water fever occur, but more amongst Babus than Europeans. The Babu is an intermittent taker of quinine, and will take it while he has fever and for a short period after, but can rarely be convinced of the necessity of continuing the treatment. He takes sufficient quinine to prevent the defensive capabilities of the spleen, etc., developing, but not enough to effect a cure, and undoubtedly a malarial infection can be cured by quinine, more easily if relapses have not occurred, but the course necessary requires considerable intelligence and perseverance on the part of the patient. The subject of malignant tertian getting repeated attacks of fever, partially held in check by a temporary taking of quinine, is asking for serious trouble. The value in controlling malaria by the systematic administration of quinine to troops or labour is disputed, but it is presumable that what is so successful in the individual should be beneficial to the mass. It is extremely difficult, however, especially with children, to ensure that all will take quinine regularly in sufficient dosage, since those engaged in manual labour appear to require a larger amount than those in sedentary occupations, and if the dosage be insufficient more harm than good is done. An experiment on one individual garden is of little value, as in this case a special supervision is exercised which it is impossible to give to every garden in a practice. It is an uneconomical and troublesome way of tackling the problem, and should be abandoned for more thorough measures.

The effects of malaria on the labour force in this district are those common to most parts of Assam, but as the coolies are, on most of the gardens, of a good type and garden-born, the adults show a fair amount of immunity and resistance, and the mortality and sickness is less than it would be among an inferior labour force. The most disastrous effect is the check to the increase of population, caused by miscarriages, still-births, and deaths of infants and children from fever and convulsions. The total loss to the tea industry from these causes must be enormous, for one garden-born coolie is worth many expensive immigrants. Anæmia is a prominent sequela of malaria, and although hookworm usually receives all the blame, it is undoubtedly the dual infection with these two parasites which makes anæmia such a scourge in Assam. Loss of efficiency due to malaria is not confined to the manual worker, and it requires emphasizing that impairment of memory, judgment, and decision is a frequent result.

The period of malarial infection appears to be more limited than in most districts, but during that period it is intense. Its duration is naturally dependent on conditions being suitable for the anopheles to breed, and the chief factor affecting these conditions is rainfall. At a time of year, for instance, when hot dry winds blow, and all surface water is dried, breeding-places are abolished, and fresh infections are few, if any. It may be broadly stated that, from November to May inclusive, fresh infections are negligible, but this period may be lengthened or shortened according to the fall of early or late rain. Apart from suitable breeding-places, the drop in temperature after the middle of November not only makes the adult mosquito sluggish, but very much delays the larval stage (I have kept arvæ actively alive for over two months), thus much diminishing the chance of survival. The lower temperature has also probably a retarding, or even inhibitory effect on the development of the parasite in the mosquito. The land actually under tea cultivation is unusually unfavourable for the breeding of any kind of, mosquito. The water either runs off quickly, or is absorbed by the sandy soil, and as the drains are not backed up they do not hold water long enough for the mosquito to mature. This lack of facilities for the insect is fully compensated for by the intersecting low-lying land, for there it has everything in its favour. It can take its choice of water stagnant or running, muddy or clear, shaded or unshaded, and undisturbed by the breeze, can breed in profusion. At least eight species of anopheles are found in this district, but fortunately for the survival of the human, Dr. Strickland informs me that most of them are not very capable carriers. We require, however, a good deal more knowledge than we at present possess as to the malaria-carrying potentialities of the various species in Assam, and this is one of the questions requiring more research work. The wet cultivation of rice in this district is, as in many other countries, one of the biggest obstacles to the solution of the malarial problem, and sooner or later the necessity of prohibiting such in contiguity to lines and bungalows will come to be realized. As far as Europeans are concerned, the damage due to malaria can be, and is, very considerably minimized by prompt treatment, the use of quinine, and the protection afforded by wiring and electric fans, but these measures are impossible in the case of the coolie, and the only practicable way to prevent the at present large loss of labour by death (especially children), ill health, lowered vitality, and reduced birth-rate, is to tackle the mosquitoes responsible. At one time this appeared a somewhat hopeless proposition in a country so much under water as Assam, but it is extraordinary how limited in reality the breeding-places are, how much water is unsuitable, or larvæ destroyed or washed away. To obtain economic results, it is necessary that each estate should be investigated individually, and a knowledge first be obtained of the species, habits and comparative malignancy of the local anopheles.

Chapter IV.

NOTES ON THE INCIDENCE OF MALARIA ON A GROUP OF ESTATES IN SOUTH SYLHET.

By

HUGH SMITH, M.D., B.Ch.

The seasonal incidence of malaria is fairly constant, and does not vary much from year to year in spite of the fact that the rainfall varies considerably.

From June to October the index is at its height, dropping at the end of October, and rising again in December with the advent of the colder weather. Catarrh is also rife at this season, and the rise of temperature accompanying it is often erroneously attributed to malaria, as it is a difficult matter to examine the blood of every patient, but undoubtedly there are many cold weather relapses as well as primary infections, carriers being in evidence throughout the winter.

The fact too that this is the recruiting season for labour should be taken into account, as many new arrivals are not immune.

While the influence of the rainfall is an important factor in regard to malaria, the distribution must be taken into consideration, and also the nature of the soil, as in a sandy soil the rain is quickly absorbed whereas in a clay soil the ground may remain moist for many days after the rain has ceased.

There is evidence to show that the price of food, and variation of wages has an effect upon the malarial incidence, possibly due to the fact that a poorly nourished coolie is less able to resist an attack of malaria, and being below par, will not recover so quickly.

The incidence of malaria in relation to jungly conditions is not marked, provided there are no streams in the immediate vicinity and the jungle is left undisturbed, but if coolie lines have been drained, and cleared of jungle, and it is allowed to grow again, there is usually a marked increase in the number of cases of malaria.

In regard to paddy growing in the vicinity of dwelling houses, it has been noted that there are fewer cases of malaria where the low-lying land has been brought under cultivation which is probably due to the presence of numerous small fish which feed on the mosquito larvæ

The manufacture of bricks in the vicinity of dwelling houses is indirectly the cause of increased malaria infection, as the pits formed in order to extract the clay, will remain for years as a breeding ground for mosquitoes. Injudicious draining should also be avoided, as in many instances badly planned drains simply act as breeding grounds.

Of the many types of coolie houses, those constructed of mud appear to be the favourite haunt of the mosquito, as there are many little crevices in the walls in which it finds a secure resting place.

Very few mosquitoes are to be found on the ceiling of a coolie house, as they are driven away by the smoke resulting from the cooking which is done over an open fire in the house, and this deposits a slimy soot on the ceiling.

The favourite haunt of the mosquito is in the little lean-to which the coolie builds against the side of his house, and in which he keeps his cattle

Chapter V.

NOTES ON THE MALARIA INCIDENCE ON THE GARDENS OF THE ASSAM FRONTIER TEA CO., LTD., FOR THE YEARS 1921—1925.

By

D. P. WILLIAMS, M.A., M.R.C.S., L.R.C.P.,

Medical Officer to the Assam Frontier Tea Co., Ltd., and Budla Beta and Bokpara Divisions of the Budla Beta Tea Co., Ltd.

(The figures which form the basis of this report are, for the sake of convenience, taken from the gardens of the Assam Frontier Tea Co. only.)

Physical Features.—These tea gardens are situated in the most northerly part of the tea-growing districts south of the Brahmaputra. The northern and north-western boundary of that part of this great river basin in which these gardens lie is the Brahmaputra itself, the lower ranges of the Himalayas lying beyond it; while the eastern and southern gardens are confined by the Naga Hills. Except for these limits the gardens have no natural boundaries except the line of the Dibru-Margharita section of the Dibru-Sadiya Railway on the south-east and south-west. Jungle separates the tea-growing area from the south-westerly bend of the Brahmaputra on the west and from the Naga Hills on the east. This area is commonly known as the Doom Dooma District, with the small town of Doom Dooma about its centre.

The Dibru-Sadiya Railway intersects the district from north to south, and the gardens lie on the east and west of the line and close to it. In some places where the railway bund is high, and the natural drainage blocked, this railway must have an important bearing on the malaria incidence. The height above mean sea-level is roughly 400 ft. at Doom Dooma, rising to 410 at its most northerly point. The average rainfall and temperature records are appended.* Subsoil water is found in March at a depth of about 30 ft. The area taken as a whole consists of plateau on which the tea is grown, surrounded or intersected by 'pathar' or bheel land, lying at levels varying between 5 and 25 ft. below the plateau. These pathars or bheels vary much in width and depth, some being only a few hundred feet across, others over a mile; some have more or less a clean cut stream running in them, with marsh on each side and very high banks, 25 to 30 ft. below the general tea-level; others are wide expanses of land more or less dry, at least in the cold weather, at only a few feet below the high level, and may have very gradual sloping banks. In these latter bheels rice is grown. This varying physical aspect has an important bearing on the malarial incidence on different gardens. Along the bottom of the steep banks the water emerges clear and is favourable to the breeding of two of our malaria-carrying mosquitoes—namely *A. maculatus* and *A. minimus*, especially where such banks are clear of jungle. These two species shun the contaminated water of the open broad low-lying pathars, but select the clean water of deep and narrow bheels. This fact will account for the difference of the malarial incidence on contiguous gardens: for the main population lives along the sides of these various types of bheels.

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* Appendices are filed for reference in the office of the I. T. A., Calcutta. Such items will be marked in the succeeding pages (I. T. A.). It is greatly to be regretted that Dr. Williams' beautiful graphs cannot for reason of space be published. They should be obtained from the I. T. A., Calcutta, and consulted.—C. S.

The district is alluvial and its soil rich, once having been the bed of the main river.

With these endless bheels and waterways and natural water reservoirs, an optimum rainfall and temperature, and a crowded population the district is a highly malarious one, as would be expected. A consideration of the map will make these points clear (*see* the attached map) (I. T. A.).

The gardens of which the writer is in medical charge, are in two main divisions, called the upper and lower, with one garden half-way between the two groups.

The physical features of all these gardens are roughly identical, i.e., plateaux of tea, intersected or surrounded and separated from its neighbour gardens, by pathars or bheels as described above; the people are settled out on all the gardens in much the same way, that is partly in regular lines on the garden itself and partly in bustis in scattered, irregular, groups of houses varying in distance from 1 to 4 miles away from the factory. It is the policy of this Company to allow the coolies to settle out according to their own ideas and customs, depending on the caste prevailing in a particular garden, rather than to settle them in organised lines on the garden itself. It is difficult to estimate which is really the healthier, as many factors have to be taken into account in estimating the incidence of malaria respectively; actual hospital statistics show the busti population to be the healthier, as far as malaria is concerned, in the proportion of 7·3 or 7·4; this is gauged by hospital admission statistics; but it must be taken into account that those living a long way off do not report ill as readily, nor is a person lying sick discovered as easily, as those living near the hospital in regular lines more or less under control and daily inspection; this is especially true of malaria in children and many deaths occur in children in the bustis who have never been seen by the medical staff.

The busti settlements are usually nearer open water and the streams of the pathars and bheels, but this danger is more than balanced in the lines by crowding and the extra difficulty of effective drainage. But after taking all facts into consideration figures seem to show that these out-lying settlements are healthier than the garden lines, and probably the Company's policy to settle out as many as possible, with the gradual elimination of the garden lines is a wise policy and makes for happiness and content in the labourer; he becomes, in fact, a small farmer. But without question it makes efficient medical work and medical administration and organisation with the limited staff at our disposal much more difficult. To put it quite frankly, the modern tea garden labourer is not under medical control; he looks on the garden no longer as his home, but only as the place where he earns his living, and the work over for the day he goes home, exactly as the British worker regards, we may say, a factory in his town; if ill, he does not come to work, but obeys the simple biological law instinctive in all animals and retires to his darkest corner. Hence the great difficulty that the modern medical officer has to contend with indirect treatment of illness and personal medical prophylaxis, and hence the enhanced need of a broad, all-embracing prophylaxis in combating malaria, in the carrying out of which the labourer shall be in no way directly concerned.

The four 'upper' divisions are about fifteen miles due north of the lower group, with one garden, Sookerating, midway between the two. In addition an isolated garden, Dholla, lies in the 'Political' area near the Brahmaputra some seven to eight miles north of the upper group. In the accompanying map (I. T. A.) the gardens are marked by a red ring. From the appended statistical tables it will be seen that the upper gardens are on the whole less healthy as far as malaria is concerned than those of the lower group and malarial infection is the common factor that, judged over a number of years, seems to determine the mortality and sick-incidence rates of the gardens; consequently we must examine the physical features to account for this.

It would appear that where a garden is in close relation to deep and narrow bheels fifteen to twenty-five feet below the tea area, especially if a small well-defined stream run through the bheel that garden is highly malarious; and conversely an estate that merges gradually into wide open pathars or marsh, in the absence of steep banks or clear streams, is comparatively healthy. Speaking broadly the four lower divisions conform to the second type, and the upper divisions to the first type. Statistical Table I (I. T. A.) shows the malarial incidence on the company's gardens for the last five years. The first four gardens on this list and the last one, Lainah, are in the upper group. These figures show the higher incidence on all the upper division groups, with the exception of Khobong, which conforms in type to those of the lower group; and the writer personally knows no other way of accounting for it except by a consideration of the physical features as given above.

Mosquitoes. The following note on the local mosquitoes was kindly given to the writer for publication in this report by W. H. Scott, Esq., A. R. San. I., who is in charge of Sir Malcolm Watson's Anti-malarial Operations in the Doom Dooma and Associated Companies' gardens.

"The anophelines found on a group of tea gardens with an average spleen-rate of 70 per cent have been *A. minimus, maculatus, barbirostris, sinensis, fuliginosus, leucosphyrus, kochi, gigas, rossi* (Giles). Work is being carried out to control the breeding places of *minimus* and *maculatus*. When the subsoil water rises and breaks through on the lower edges of the bheel, the clear water fills up small cavities and unless shaded by heavy vegetation forms an ideal breeding place for *maculatus*. These small pools, constantly being fed by seepage water, overflow and join up in a regular stream in which *minimus* abound. Where the bheel runs back into the high land in the form of a re-entrant or bay a greater volume of seepage is found, which naturally forms a more dangerous breeding ground. The ideal system to control these carriers is by intercepting the spring water at the bheel edges by subsoil drainage at a depth of four feet. At anything less than four feet the pipes are liable to be choked by the roots of bheel vegetation. The water from the subsoil pipes is carried off by a central open drain. This system can only be carried out in small areas owing to the great cost; but on a large scattered area open earth-work, consisting of contour, herringbone, and central drainage, can be done. The contour drain should not be more than six inches wide at the bottom; this keeps the water highly concentrated making the drain self-cleansing and often prevents direct sunlight reaching the water, making it less favourable for *maculatus* and *minimus*. A mosquito survey, made on a wide flat pathar with a spleen rate of 8 per cent, revealed *fuliginosus* and *sinensis*. This survey was made in August; there was an average depth of four inches of clear still water and where the soft mud was disturbed it gave off an unpleasant odour. The particular pathar examined was intersected by a road, and at the culvert there was a regular stream caused by the invert being too high. These places had the appearance of ideal *minimus* breeding grounds, but the water was not of a purity essential for it. A careful search was made, but only *fuliginosus* was found in the actual stream-way. It is interesting to note that of two gardens not affected by seepage water, through both of which flows a small river, one is healthy and the other unhealthy. On the healthy garden the river is free from jungle and only *fuliginosus* was found in the actual stream-way. The river on the unhealthy garden was covered in jungle, and in the stream *fuliginosus* was found, but in the small backwaters *barbirostris* was breeding in large numbers. Unfortunately this survey was made at the end of the rains, but if a close observation were made covering several months, the information would be valuable to many tea gardens" (W. H. Scott).

Annual Malaria Incidence. Table II (I. T. A.) shows the malarial incidence throughout all the gardens for each year 1921—1925. From these figures it will be seen that 1922 was a year of high incidence, and that there has been a gradual decline since in the total figures. I know of no reason to account for this high incidence in 1922, except possibly the excessive early rainfall of that year. The gradual decline is, I think, attributable to the comparative absence of recruiting in recent years, as the population has remained practically stationary and no anti-malarial measures have been adopted. In the absence of recruiting of new coolies from India the proportion of the relatively immune to those who are not gradually increases. The only other factor that could influence this decline is the gradual shifting of the population into widely scattered busti areas and the corresponding diminution in 'line' population.

Table III (I. T. A.) shows the malarial incidence by months for the five years. This roughly follows the rain and temperature curves and calls for no comment.

Statistical Table IV (I. T. A.) shows the malarial incidence on each separate garden for the five years. One or two interesting facts emerge from a consideration of this set of figures.

1. On the whole there is a small but steady decline.

2. Talup Estate, where the malarial incidence is far higher than on the other gardens of any group, is the only garden to have recruited new coolies in any numbers. On this garden malaria was almost epidemic in July and August of 1922; this is the only real malarial epidemic in the experience of the writer; it was confined to one large set of new lines or rather settlements into which a large part of the population was being transferred; hardly one house escaped infection in the new settlements and nearly every inhabitant passed through the hospital. This acute and sudden infection was attributed to the change being effected in the rains before the land immediately adjacent to the houses was levelled off; the whole area was a mass of holes from the uprooted tea, and borrow-pits, with water everywhere. The epidemic ceased on the ground being levelled off. But this garden, as a whole, shows a great diminution in malarial infection, as the figures show, owing, in the writer's opinion, to the wholesale abolition of lines in favour of scattered settlements. The same remark applies to Dangri, where the drop in the number of cases is remarkable probably due to the same cause, namely, the migration of the labour away from the garden. Hokungurie shows the same type of drop and is due, in the writer's opinion, to the same cause. In other words, other things being equal, malarial infection in a given population varies directly with its density.

These figures give only a fraction of the actual malarial incidence, for:—

1. The malaria that is seen complicating other diseases, such as dysentery, pneumonia and anæmia, etc., is not shown in the case-sheets as malaria, but as the disease of admission. Very few cases in hospital for more than ten days escape at least one attack of malaria.

2. The cases of malaria treated as out-patients are at least three times the number of in-patients.

3. A large number, especially amongst those who live in the bustis two or or four miles away are not reported at all; this is especially the case in little children, a matter referred to under mortality.

4. (*a*) The vast majority of our so-called dysenteries are malarial in origin. The typical temperature charts on admission show this, and the temperature and disease so commonly clear up under quinine. Statistical Table V (I. T. A.) shows the correlation between these diseases.

(*b*) Anæmia is largely malarial in origin. The charts of all these cases—and the number is enormous—show a malarial attack in hospital roughly every six or eight days when first admitted, which gradually diminishes in frequency as treatment goes on; quinine is ordinarily given for anæmia in these cases during the early days of treatment. No cure is possible until the system has been cleared of malarial infection. There is a drop of about 5 per cent in the hæmoglobin index at each attack of malaria and the anæmia in a garden is a good rough working guide of the malarial incidence. Without adequate daily anti-anæmic organisation to cope with it, indeed, a labour force would go down-hill and ultimately disappear.

(c) Last year the following experiments were carried out on a large scale. The following types of cases were admitted into hospital:—

(a) Normal-looking women, but wanting a rest, with children.
(b) Anæmic women—with their children.
(c) Pregnant women—with their children.
(d) Women with slight ailments—such as skin diseases, ulcers, slight wounds, etc.
(e) Orphan children.

Accurate four-hourly charts were kept. With hardly an exception all had one or more attacks of malaria in hospital—the average being about every eight or ten days—some, of course, far more frequently. A full account of this was given by the writer in a paper read at the Dibrugarh B. M. A. meeting in 1924. In the absence of accurate figures on this point one hesitates to generalise, but it is no exaggeration to any that children up to about 3 years old seem to live in a state of constant active malarial attacks, and the marvel is how so many survive. Dysentery and anæmia are the two best indices of malaria.

There are other valuable but indirect ways of estimating the prevalence of malaria and the extent of its ravages in a labour force.

Considerations of the following data suggest themselves:—

1. The children's spleen-rate. Table VI (I. T. A.).

2. Total children's monthly deaths from all causes compared with the total children's monthly deaths from malaria. Table VII (I. T. A.).

3. Still-births by month compared with the monthly malarial incidence. Table VIII (I. T. A.).

4. Children's monthly death rate compared with the monthly malarial incidence. Table IX (I. T. A.).

5. Deaths of children in the first twelve months of life. Table X (I. T. A.).

6. Deaths of children 'in the line' compared with the total children's deaths. Table X (below).

Numbers 3, 5, and 6, are valuable indices of malarial prevalence, but require a little explanation:—

Statistical Table VIII shows the monthly relation between still-births and malarial incidence. For every one hundred children born there are roughly ten still-births, abortions in the early days of pregnancy are not counted, but only those occurring after the first six months of pregnancy, as the condition of these mothers is known, being under observation, and the cause of the still-births, as far as can be accurately known, is recorded. In the vast majority of cases the causative agent is either a single acute or several small attacks of malaria in the mother, or the anæmia caused by malaria. This sequence is so common as to admit of no doubt.

Table X shows the deaths of children in the first twelve months of life for the three years 1923—1925; this table also shows the births for these three years, the total deaths of children from all causes, and the total still-births. It will be noted that the mortality in the first year of life is very high; it works out at about two hundred out of every one thousand born. This, however, is small compared with the large towns in India where the infantile mortality in the first twelve months varies according to different towns from two hundred and fifty to five hundred per mille. The cause of this mortality is again chiefly malaria, due either to repeated attacks or one single attack, or more often the cause is written down marasmus or debility at birth, which condition, again, is itself due to anæmia of malarial origin in the mother.

Table XI (I. T. A.) shows the monthly 'line' deaths in children contrasted with the monthly malarial incidence. By line deaths we mean the more or less sudden deaths of little children at night in their parent's house without being seen medically. The history of these deaths at night is nearly always the same; the child is on its mother's back during the day; after returning to the house and preparing the evening meal the mother may notice that the child is fretful and hot, or she may observe nothing; the family goes to sleep and nothing further is known until the child is found dead in the morning.

This tale is only too familiar to us, and from what we see in hospital the sequence of events is clear—fever during the day unnoticed, a rising temperature at evening, later hyperpyrexia, coma, death. The malarial history is again manifest.

The last three tables can be tabulated as follows:—

TABLE X.

	1923.	1924.	1925.	TOTAL.
Total children born (live births)	980	976	969	2,925
Total children's deaths	321	369	373	1,063
Total still-born	124	125	108	357
Total children's deaths in lines	182	224	187	593
Total children's deaths in the first twelve months of life	157	209	166	332

The mortality directly due to an attack or attacks of malaria is small statistically and is chiefly in children. Figures are appended to show this. Table XII (I. T. A.).

But to estimate the mortality from malaria is impossible directly and statistically.

We can only see its malign influence by a consideration of the following :—

1. The general lowering of health, paving the way for final dissolution under another name—in attacks of such severity as should not normally cause death. Such as :—

(*a*) Dysentery.

(*b*) Anæmia—General œdema, albuminuria.

(*c*) Sudden death from myocarditis.

(*d*) Diarrhœa.

(*e*) Choleraic diarrhœa—so called.

(*f*) Deaths of mothers in pregnancy and confinement.

(*g*) Still-born children.

(*h*) Marasmus in the first few days or weeks of life.

(*i*) Nephritis (especially in children).

(*j*) Coma—convulsion—cerebral and meningeal condition.

In our daily round the presence of malaria is only too clearly brought home to us; it induces or complicates or carries in its wake every disease that flesh is heir to. The loss to the work year in and year out is incalculable, not to mention the indirect effects of unhappiness and discontent and longing to get away.

Employment. As all coolies are roughly engaged in the same work and under the same conditions the question of malarial incidence relative to the daily occupation of the people cannot be taken into consideration here. Some, however, of the old coolies have, so to speak, retired and settling around in little farms become 'Faltoo,' i.e., work on the gardens at irregular or fixed periods of the year only. Statistical Table XIII (I. T. A.) showing the malarial incidence by lines and busti respectively, and Table XIV (I. T. A.) showing the spleen-index of line and busti population, might help us to form some idea of the relative incidence of malaria amongst typical garden-working coolies and those living an agricultural life—whether imported Indians or Assamese. From a consideration of Table XIII (I. T. A.) it is observed that the malarial incidence of lines and bustis respectively works out roughly in the poroportion of 7·4. If we omit Sookerating Garden, where the busti is too small on which to base figures for one year only, the proportion is only 7·3, which figures are identical with results obtained in working out the respective health of the lines and settlements three years ago but using an entirely different method. These figures may be of interest to the tea industry in guiding managers in selecting methods and sites in settling out their labour forces.

Caste and *District* from which recruited :—

The incidence of malaria in relation to caste is not easy to calculate. For while it is quite a simple matter to know exactly the caste and district of every malarial case admitted and this is taken as a routine—it is almost impossible to know the exact population on every garden of every caste. The sum totals which are known therefore, convey no exact knowledge, in the absence of data for working out the percentage. The writer's own impression is, and it is only a personal opinion, that the 'jungle' tribes do well and suffer less from malaria when they are settled out in more or less jungle settlement according to their own original customs and ideas; this fact is brought out in Table XIII (I. T. A.) where we see the small percentage of malaria in the bustis of Kobong (Oorangs)—Dangri (Mundas and Kumas) Hookanguri (Mundas) Hilika (Kumas and Mundas). This type of coolie suffers equally with or even more so than other coolies when settled in lines. furthermore they go down-hill much quicker under successive malarial infection than do castes like Domes, Ghassis, and 'Ooriahs' (so called). Chumars, Ghonds, Panikas and Telis (Central Provinces). Ooriahs (Dholla garden) seem the most liable to malarial infection, as judged by the standard of anæmia and adult spleen rate that is so noticeable in a pure Ooriah labour force. But there is this striking difference, that whereas a Munda or Oorang or Santal is profoundly ill in hospital with a blood index of 50 per cent, the Ooriah is gaily working on the garden with the same blood index, and makes no complaint. But the Ooriah in health will not do the same task as the Munda in health and on this hangs the successful working of a garden with a more or less pure Ooriah force; an Ooriah working with Ooriahs under an Ooriah sirdar seem to be able to withstand the ravages of malaria quite well, but mixed in gangs with 'jungli' people under 'jungli' sirdars he goes under.

While the newly-recruited coolies from any district on arrival seem equally liable to malaria, the one district that in the writer's mind stands out as unsuitable to recruit from to this area is Sambalpur (Telis and Ghonds). Not that these people seem more prone to malaria than any others, but that they stand malarial attacks badly and rapidly go down-hill in consequence, and if they do not succumb to intercurrent disease, become a burden to the garden. Those from Ganjam and Cuttack also deteriorate badly under malarial attacks and become liable to dysentery and other bowel complaints.

Sex. Men and women adults seem equally affected, which is natural as the work and environment of both is the same; the slight preponderance of the number of women affected is probably due to the stress of pregnancy. Table XV (I. T. A.). But the number of deaths from malaria direct is greater, due to the complications arising in and around pregnancy and child-birth. Table XII (I. T. A.).

Age. The greatest age incidence is in the first twelve months of life, slowly falling up to the age of ten to twelve years, and markedly diminishing after puberty is attained.

Statistical Table XV (I. T. A.) illustrates the sex and age incidence for the years 1923—1925; by children is meant those not working, up to the ages of ten to twelve years. The tremendous preponderance of malarial deaths in children is to be noted. Table XII (I. T. A.).

To compare with this, Chart 16 (I. T. A.) is inserted to show the number of children's deaths from all causes, and the number of children that die in the first twelve months of life.

To summarise these three tables for two years only, 1924 and 1925.

TABLE XVI.

	1924.	1925.
Total deaths in children	399	375
Number who died in the first twelve months of life	209	166
Number of deaths from malaria	109	93

Race. No race seem to enjoy any sort of immunity or even relative immunity, over any other. Europeans, Babus, whether Indians or Assamese, Marwaris, etc., all are equally infected. European ladies, in the writer's experience, are the least liable to actual malarial attacks, not because of any immunity, but because they take greater care of themselves than the man. One sequel, however, of malaria is only seen in the higher and fair-skinned races, namely, black-water fever; the coolie seems immune to this.

Types of Malaria. The writer has done no consistent microscopic work on this subject to enable any statement to be made as a fact based on actual figures. But many estimations, however, have from time to time been made over given periods in separate hospitals by a consideration of the temperature charts. While generally speaking, there is a small relative increase of the benign tertian in the early rains, the proportion throughout the year has worked out roughly as 70, 26 and 4 for sub-tertian, benign tertian and quartan respectively. This method of calculation is open to many objections as the influence of quinine and mixed infections and other complicating disease all confuse the picture. One other fact that has struck the writer is that in the European newcomer the first attack is generally one of benign tertian, gradually merging into the sub-tertian with longer residence.

Prophylactic Quinine. In this Company prophylactic daily quinine or rather Cinchona Febrifuge is given to the non-working children only, and only in the lines and not in the bustis. It has been given for the last two years only and that for the eight rainy months, April to November. It is given in liquid form followed by a small lump of native sugar. The daily distribution of this prophylactic quinine is attended with great difficulty; to avoid dislocation of labour and the assemblage of women with their children in the morning or during the working hours it has to be done in the lines in the early evening when the day's work is over; hence the impossibility of giving it when the days are short. The Doctor Babu and his staff go into the lines and give it to as many children as they can collect around them, noting down the number, and at the end of every month the percentage of the number to whom given to the total number of children in each line is worked out. Considering the great difficulties attending such a method of distribution, the figures 61·67 per cent for the whole Company is good. Some children run away on seeing the Doctor Babu or compounders approach; some are not there, being away on errands, collecting firewood, or tending cattle and the paddy-fields. Again, with the very limited garden medical staff it is quite impossible to attempt to give this quinine to more than two lines per day, and if the hospital work happens to be heavy not even two lines can be visited. The bustis are quite out of the question. So if each child gets prophylactic quinine only three times a week, it is the utmost one can do. As coolies do not turn out to work as early as they did, it is too much to ask the garden manager to collect all the garden women with their children in one place every morning so as to ensure the proper administration of quinine under adequate European supervision. Unless under European supervision, this prophylactic distribution is a failure and must be so, and it is a doubtful point whether the loss in the working hours thus caused every day would be compensated by the smaller malarial incidence, especially in these days, when with the shortage of labour, even half-an-hour lost, is a matter of prime importance to the industry. And to ask the European staff to give an extra hour's work in the lines at the end of a long day's work is to my mind also impracticable, especially when the lines or settlements are a long way off and widely scattered. It must also be remembered that the present garden coolie is not under the same garden discipline as a few years ago, and in the light of the average coolies' ideas and predilections we must still face the hard fact, however unpleasant and discouraging it is, that the less the medical work of every sort whatsoever on a given garden, the more popular this garden is; and, as every wise medical officer knows, the medical work on an estate is limited not by his own inclinations, but by a judicious estimation of the prejudices of the labour force. The question of prophylactic quinine has been adverted to at length not to discourage it, but because there seems to be much loose thinking on the subject, not only by laymen, but also by some medical officers, who possibly ignorant of the inherent difficulties surrounding the question, and the dislocation of labour and time involved, the great cost both directly and indirectly, and the possible upset of a labour force, light-heartedly recommend it. With the number of gardens to be visited and the distances to be covered, the medical officer cannot properly supervise it, and herein lies the weakness of the system—at least in the tea industry.

In any case the absolute value of quinine as a prophylactic is difficult to determine. Influenced by his own war experience, the writer does not believe in quinine prophylaxis in adults as a business proposition at all; its value is very doubtful, but in the absence of accurate figures and accuracy in this respect is most difficult to get, one can hardly dogmatise; published figures based on apparently accurate knowledge and statistics seem to lead to diametrically opposite conclusions. The writer has more faith in its value in children; this is, however, only a personal opinion based on general observation, and is not supported by accurately compiled figures extending over some years and balanced by controls. The incidence of malaria on some gardens already referred to makes a curious graph in the last five years and by special pleading a special case could be made out for the value of anti-malarial measures of any sort, when in fact none had been carried out.

Honesty of mind, though essential, is very difficult in this matter. Though the writer has not given figures, the mortality of women in pregnancy labour and after labour tells the same sad tale; it will be sufficient to bring it home to any one to state the terrible fact, as seen from the figures of five years, that for every hundred children born six mothers die, and in the vast majority of cases malaria is the underlying cause.

Some of the tables of figures given in this report may seem out of place in a malarial survey; they may be unusual and perhaps generally unknown; but the writer feels that they throw much light on the general malarial incidence, and if they help in estimating the ravages of this 'million murdering death' and so to a determination to eradicate it they have not been compiled in vain.

The figures deal largely with women and children. Whenever in our history those of our race have been collected together in times of stress, the cry of 'save the women and children' has always quite naturally, somehow, brought out the manhood in us. We are raising the same cry now.

Before concluding, the writer will, if he be allowed, offer one word of warning. The tea-growing areas and their grants, by virtue of their great extent and diversity of physical features of even one estate, the diversity of peoples and castes and their deep ignorance and prejudices and the widely scattered and even constantly shifting population, and the essentially agricultural life of the people, the tea industrial areas present great difficulties to the anti-malarial worker. He has no real parallel in the past for comparison and no sign-posts or landmarks to guide or warn him; the difficulties of the task ahead are only too obvious to those familiar with the districts, the ultimate result no man can tell. Consequently a caution and gradual policy seems to be indicated at first; it would seem that after a gradual anti-malarial survey has been made of the district, it could be wise to confine the actual anti-malarial work at first to one estate only or possibly two, if contiguous. This work should be absolutely thorough, the best in the light of the knowledge acquired in the general survey; accurate records of all kinds pertinent to the matter should be kept, and guided by these results and by these results alone, compared with and augmented by similar work in other districts, the worker could proceed to wider measures; one worker looks liable to be overwhelmed in the tackling of this huge problem over, say, ten scattered gardens in one company.

Above all, knowledge should be pooled and placed at the disposal of all workers, and this can only be done by the whole tea industry uniting for this one object. It must be borne in mind that no money can buy the skilled anti-malarial worker at the present time; the Indian Government through its Services cannot supply them, however wishful it may be to help and however alluring the prospect. Consequently, it behoves the industry to use the few available to the very best purpose in the interests of the industry as a whole by a wise combining of forces, and by placing the whole direction of the work under a central administration.

In addition to the statistical tables and graphs referred to in the text, there are appended (I. T. A.):—

1. A graph showing the relationship between malaria and black-water fever.

2. The general birth and death percentage on all the gardens. In the birth percentage still-births are, of course, not counted; and in the death percentage all deaths are recorded from infants, who have breathed, to those of extreme old age, all of any sort whatsoever who live in the garden grants if agreement or faltoo workers.

3. Figures for each of the tea garden separately, showing :--

(*a*) The malarial in-patients for 1925.
(*b*) The malarial in-patients by line and busti respectively for 1925.
(*c*) The spleen-index of children by line and busti for 1926.
(*d*) A map of the district. Tea gardens under the writer's medical charge are surrounded by a red line; those coloured blue show the gardens on which one or more cases of black-water fever have occurred in the last five years.

I wish to express my thanks to W. E. H. Grayburn, Esq., the Superintendent of the Gardens, from whose hospitals these figures are taken, for his kind permission to use them and for much valuable help in many ways.

To Sir Malcolm Watson and J. Pringle, Esq., for their kind assent to the publication of Mr. Scott's note.

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PART III.

THE PERIODIC INCIDENCE OF THE DISEASE.

By

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assisted by

K. L. CHOWDHURY, M.B., D.P.H.

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SUMMARY OF PART III 63

Enquiry into the Periodic Variations of some Malaria Indices.

An enquiry of this nature sometimes affords useful information concerning the genesis of malarial epidemics. For example, if it be found that malaria case-rate decreases during the rainy season one may conclude that the stream-breeding anophelines are an important factor in the situation, and one is immediately armed to a certain extent for dealing with it; the combat is with the stream, the back turned to the swamp. But even if no such useful information be forthcoming, data are certainly obtained for empirically forecasting the malaria future in the locality and that, as Gill (1923) has pointed out, is of great importance in preventive work.

The results of our enquiry in these matters will be submitted in two sections dealing respectively with (A) seasonal data, (B) annual data, first attempting to establish some correlation between the malarial indices and the meteorological conditions, and then investigating some inter-correlations between the various indices.

The conclusions from such a study are, of course, subject to the 'errors of random sampling.' However, our compilations for wide areas and long periods *having been found not to vary,* and being corroborative *inter se* one may assume that the error has been reduced to a minimum and we may therefore regard the figures as a standard for assessing the limitations to the use of similar compilations over small areas and short periods.

SECTION A.

The detailed results have been tabulated in the Appendices, but condensed analyses are now submitted under the heading of the several indices.

(a) (b) *The spleen-index* (*see* Appendices III A and B).

For the whole of Assam, for the two half-yearly periods, the analysis of our data in the detailed survey of 1924 gives approximately the same reading. A minimum of 5,932 children was examined, and the readings of the quarterly analysis give the same result (minimum numbers of children in any quarter, 2,525).

For all practical purposes then the spleen-index in a large area is not affected by seasonal influences and therefore any change which the index undergoes may be accepted as evidence of a corresponding change in some factor inducing malaria which is not of a seasonal nature, for example, a swamp being drained.

The variation in the monthly records for the whole of Assam which one sees in the compilations must be due to local disturbances the effects of which have not been masked by examinations over longer periods (minimum number of monthly examinations, 836).

These conclusions emerge from both the analysis of the index of only the garden-born children, and of the garden-born children combined with the 'country-born' recruits.

Of the smaller areas in which the analysis was made, the two valleys showed no definite differentiation in the index in either of the two half-yearly periods (children examined, 1,250), but there was definite variation at the four seasons and still more in the monthly figures.

The districts showed a pronounced variation even between the half-yearly periods, probably due to the action of some local non-seasonal influence.

(c) *The parasite-index* (*see* Appendix III).

The parasite-index, unlike the spleen-index, showed rather a wide variation between the two half-yearly periods. In the August to January period it was 9·4 per cent and in the February to July 15·4 per cent.

This experience was repeated in greater or less degree in nearly every one of the smaller areas (minimum of 56 children) in which the index was computed.

If we examine the corresponding figures for the quarterly periods (obtained from a minimum number of 323 children), we find the lowest parasitage in the November to January quarter, higher and higher in the next two quarters, and then a sudden drop in the autumn.

There was no apparent correlation in these conditions with climatic conditions.

In the few smaller areas for which computations were made, the respective figures became irregular and not conformable to the standard set up above.

The same reservation must be made with regard to the monthly figures, which, however, generally show a rather significant drop in July which is sustained through autumn and winter.

The parasite-index, then, shows a definite variation at different seasons, which is not apparently correlated with climatic conditions.

(d) *The hæmoglobin-index* (*see* Appendices).

This index was computed for only a few estates. In them it was found that the average hæmoglobinism of the children during demi-annual or quarterly periods did not vary to any appreciable extent, the maximum difference being 6·5 per cent (half-yearly) and 7·5 per cent (quarterly).

(e) (i) *anophelism : the anopheline-index.*

The total anophelines caught as larvæ in 1924 on all tea gardens during the 'rainy' half of the year were more numerous than during the drier months, which statement probably reflects the natural state of affairs, for collecting during the rains is attended by some difficulty and discouragement and in the collection of mosquito larvæ the human 'equation' is a considerable factor and comparisons odious. An analysis, however, of the larva catch all over Assam made during the various quarters of the year showed that during the *active* monsoon months larvæ were no more prevalent than during the dry half of the year: it is the quarter September to November that is most prolific, a fact which confirms public opinion in the matter.

(e) (*ii*) *anopheline species-index.*

We will now consider in conjunction with the records of previous observers, the evidence with regard to each species collected by us in both the preliminary survey (1923) and the detailed survey (1924).

fuliginosus.

Fry (1912) (1914) found this species in enormous numbers in winter, gradually increasing from August through the autumn months and diminishing again in the hot dry months.

Chalam and Young (1922) from May to November 1921, found 52, or about 2 per cent of their total adult catch, and in the winter months of 1922-23, 8, or about 14 per cent.

Watson (1924) during the winter months January to March captured an enormous number (2,114), of adults, or about 94 per cent of his grand total.

Strickland (1924) (September to November) in the preliminary survey, found 387 breeding places of either *jamesi* or *fuliginosus*, i.e., about 22 per cent of all, and as only 1 *jamesi* bred out as against 48 *fuliginosus*, it may be taken that the catch was mainly *fuliginosus*.

In our detailed survey (1924), from June to November, *fuliginosus* with *jamesi* occurred 178 times and in the dry half of the year 188 times, findings which are quite consonant with the above-mentioned records. Further, the quarterly records of our survey strongly confirm other observations, thus :—

Periods.	*fuliginosus* or *jamesi* breeding-places.
June to August	43
September to November	135
December to February	132
March to May	56

They are specially in accord with Fry's notice of the 'great autumn and winter prevalence of the species,' and its decrease in the hot months.

It must be observed that the rains is its least favourable season, a finding which contrasts with McCombie Young's report that in Shillong the species is commonest during this season.

To summarise, we may say that *fuliginosus* is, in the Valley essentially a cold-weather species, and as it occurs in Shillong chiefly during the rains, it is probably because the rains' temperature is its optimum.

jamesi.

This species (one specimen) was captured by Strickland (in 1923) in November, and two more were bred out from larvæ (in 1924) in June. However, some of the larvæ identified as *fuliginosus* may have been of this species, as a diagnosis was not made. Nothing can be said of its seasonal prevalence.

aconitus (*albirostris*).

Fry (1912) (1914) says that this seems to be more prevalent in the spring.

Strickland, in the preliminary survey in September to November 1923, found 34 of its breeding places or about 2 per cent of the total.

In our detailed survey we found 11 during the rainy half-year and 19 in the dry half.

The following were the quarterly rates :—

Period.	*aconitus* found.
June to August	2
September to November	9
December to February	10
March to May	9

It would appear then that, except for the rainy quarter when it was comparatively scarce, it is about equally prevalent over the year, but the numbers are really too small to be of much value.

funestus (*minimus*).

Fry (1912) (1914) expressed the opinion that this species seemed to be more prevalent in spring, but that 'they are so scarce that no accurate data of their seasonal variation can be given'.

Chalam and Young (1922) found 46 adults or 86 per cent of their total in December and January; and 1,077 or 45 per cent. from May to November.

Watson (1924) captured 137 adults or about 6 per cent of his total in January and February.

Strickland (1924) found 102 breeding-places (i.e., about 6 per cent of the total) in September, October and November.

Christophers (1925) obtained it in considerable numbers as late as November, after which it 'along with other species, diminishes.'

The above records show that there is no month in the year when it is not plentiful though it cannot be said which months are the most prolific. It is indeed curious that with regard to this species which has been so universally condemned so few seasonal data have been collected.

In our main survey we found 8 breeding-places during the rainy half-year and 13 during the drier half, too slight figures to be of much value, but sufficient perhaps to indicate that the species has no very marked seasonal variations in prevalence, with a tendency to an increase in the dry half of the year.

funestus finds no place in Shillong.

barbirostris.

Watson (1924) captured only one *barbirostris* from January to March.

Strickland (1924) from September to December found 334 breeding-places, about 20 per cent of his total, and 135 more of either *barbirostris* or *hyrcanus*.

In our detailed survey we found 151 *barbirostris* breeding-places during the rainy half-year, and 64 during the other half.

The quarterly records being as follows :—

Periods.	*barbirostris.*	*barbirostris* or *hyrcanus.*
June to August	91	18
September to November	60	43
December to February	24	47
March to May	40	18

They show that the maximum prevalence is at the onset of the rains, there is a decrease at their cessation, the minimum rate is during the cold weather and with the onset of the hot dry months the rate slightly increases.

We may summarise that *barbirostris*, a pool breeder, is essentially the species which is most prevalent during the rains and conclude that as the pools dry up in the autumn and winter its numbers decrease, the 'chota-monsoon' again bringing an increase.

hyrcanus (*sinensis*).

Chalam and McCombie Young (1922) in the period May to November took 49 adults of this species or about 2·5 per cent of the total.

Watson (1924) from January to March got 73 adults, or 21 per cent of his total.

Strickland's corresponding figures in the preliminary survey (September to November) were 325 as larvæ or about 20 per cent and in addition there were 135 which were either *hyrcanus* or *barbirostris.*

In our detailed survey during 1924 we obtained 484 records from June to November and 308 from December to May, so that it appears that the rainy half of the year is more favourable than the other half to this species.

However, the quarterly records in the subjoined table :—

Periods.	*hyrcanus.*	*hyrcanus* or *barbirostris.*
June to August	169	18
September to November	375	43
December to February	224	47
March to May	84	18

show that it is in the months just after the active rains that the species is most prolific : during the rains it is comparatively scarce. It is also quite a cold weather species, but when the hot dry months supervene it decreases rapidly: it is with the onset of the rains that it starts to increase.

For comparison, it may be mentioned that during the rains in Shillong *hyrcanus* is reported to be one of the commonest species (Shortt and McCombie Young).

hyrcanus, like *fuliginosus*, usually called a swamp breeder, may be contrasted with this species, from which it differs in finding its optimum condition in autumn instead of winter. Nevertheless, it must not be forgotten that it is very prevalent also in the rains, and in the cold months.

In Shillong, the difference in the climate makes it appear most prolific during the rains, the conditions there in autumn approximating closer to those of the valleys.

karwari.

This species has not been recorded by other workers in Assam; but Strickland in the preliminary survey found 17 breeding-places in which the larvæ were either *maculatus* or *karwari.* Two of them eventually turned out to be *karwari,* and some of the others may have been.

leucosphyrus.

During the preliminary survey *leucosphyrus* was taken on five occasions.

There have been no other records of it from Assam.

kochi or *tesselatus.*

There are no records of these species by other Assam workers.

Strickland (1924) in the preliminary survey (September to November) found 84 or about 5 per cent of his total catch to be of these species. Of them 20 turned out to be *kochi* and 2 *tesselatus,* the remainder equivocal.

In the detailed survey, from June to November, 37 were captured, and from December to May, 9, so the cold and dry hot months seem to be inhibitory to them.

The quarterly records were as follows:—

Period.	*kochi* or *tesselatus.*
June to August	17
September to November	20
December to February	3
March to May	6

which confirms its rarity in the cold months and in the hot dry months, and its greatest prevalence in the rains and just after the rains.

kochi or *tesselatus* then seems to be dependent on the rainfall. It, however, does not appear to decrease so rapidly in the autumn as does *barbirostris,* the common rain-pool breeder.

rossi.

Fry (1912) (1914) stated that this species * exists in Bengal in greater proportion in the spring months and during the rains than in autumn.

We can find no record of its capture by any of the Assam workers except McCombie Young (1922) who notes that it was chiefly found in the spring months. It is possible, however, that McCombie Young's reference was to the common Assam species *vagus,* and that *rossi* is not an Assam species at all.

pallida (*fowleri.*)

Fry (1912) (1914) reported this species (from Bengal), but it has not been found by the Assam workers.

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* Probably including *vagus.*

maculatus.

McCombie Young (1922) stated that this species in the larval state was found plentifully chiefly during 'dry-weather' conditions.

Chalam and Young (1922) found *maculatus* in October and November 1921, and in 1922 from April 'onward'.

Watson (1924), on the other hand, obtained many adult *maculatus* in 1924, and these from January to March, about 3 per cent of his total catch in houses being *maculatus*. He also found many breeding-places.

Strickland (1924) reported the catch of 17 larvæ of either this species or *karwari*, i.e., about 0·6 per cent of his total catch during the autumn months, September to November.

In the detailed Assam tea garden survey over 1924, we only obtained one larval specimen of the species, and that in April.

It will therefore be seen from the above account that all the year through, except during the rains and December, *maculatus* has been reported by one author or another thus:—

	Watson.	McCombie Young.	Chalam and Young.	Strickland.	Strickland and Chowdhury.
January	X				
February	X				
March	X	X			
April		X			X
May		X			
June					
July					
August					
September ..				X	
October			X	X	
November ..			X	X	
December ..					

While December has thus drawn blank, nobody has reported looking for it then: during the rains its absence may be accepted as a working hypothesis, especially as, from what is known of its biology and especially its liking for pure spring water, its normal breeding-places would not at this season be in a favourable condition (*cf.* Perry (1914) who found a similar state of affairs in the Jeypore Hill Tracts). Attention, however, must be drawn to the fact that in Shillong while the species is most and very prevalent during the hot dry months [as one of us (C. S.) has also found in another submontane region, viz., the Kurseong Subdivision], yet it is prevalent also during the rains, a fact which has been noted by Shortt (1924) and McCombie Young (1924): but from the onset of the rains till their cessation and then on into the cold weather the species gradually decreases.

The contrast between Shillong and the valleys makes it possible that the flush of the rains is not such an important factor as has been thought. Temperature may be a more important factor.

The almost inverse difference in the seasonal prevalence of *maculatus* in the valleys and in Shillong has, it may be said here, justified the design in this report of only considering the Valley conditions in connection with the tea garden malaria.

To summarise, one may say that *maculatus*, which is a spring-water breeder in nullahs, seems to occur in the valleys at all times of the year except during the rains, the spring appearing to be its most prolific season. The flush of the rains is generally considered a powerful factor in its natural control though curiously in Shillong the rains seems to be a time when it is very prolific: and temperature may possibly prove to be a more important factor than the amount of rainfall.

gigas.

This species has been generally presumed to be a cold-climate one and a hill-dweller only, but Watson (1924) has found it in the adult state in the valley during the cold weather of 1924. No other author has found it.

We did not find it during either the preliminary or the detailed survey.

In Shillong, McCombie Young (1924) has pointed out that this is a 'cold-weather species', though specimens may be found up to June, so the conclusion is justifiable that it would probably not be found in the valley at any other time than the cold weather. Some specimens of larvæ which one of us (C. S.) took down the hill in April 1923 expired *en route* though some *hyrcanus* in the collection survived.

gigas then is a cold-weather species having a correspondingly longer 'season' in Shillong than in the valleys. In Shillong, during the rains, probably it is the flushing out of its breeding-place which eradicates it.

umbrosus.

Christophers adumbrated the ultimate discovery in Assam of this species, and Strickland during the preliminary survey found it. This was in the month of October and more were captured right up to November, in all 29 or about 1·7 per cent of the total catch.

No specimen was caught by us in the detailed survey of 1924.

In view of the habits of this species, which is a jungle-pool breeder, its chief prevalence is probably during and just after the rains, the above statement of its capture, however, being the only evidence so far in support of this probability.

jeyporiensis.

Fry (1912) (1914) did not find this species in his Bengal surveys.

Nor did any author writing in the Assam surveys record it till Strickland (1924) noted the capture of one only in November 1923.

Dr. Meek has since sent a number of specimens, all caught in August of 1924 or 1925.

These data, of course, do not enable us to make a surmise as to its seasonal proclivities.

aitkeni.

Neither Fry in the Bengal surveys, nor any of the Assam workers have reported this species, probably because they did not look for it in forest streams.

Strickland (1924) found it on four occasions during September to December 1924.

In Shillong, McCombie Young says that it does not appear till the end of the cold weather.

culicifacies.

Fry (1912) (1914) reported that this species seemed to be more prevalent in the spring months, i.e., about March to June.

McCombie Young (1921) chiefly under 'dry weather conditions' from February to May found *culicifacies* breeding plentifully.

Chalam and McCombie Young (1922) after the break of the rains found larvæ breeding scantily, while from May to November 1922 they captured 31 adults or about 1 per cent of their total catch in houses.

Watson (1924) in January to March did not encounter this species.

Strickland in the preliminary survey found no specimens of this species, either adult or larva, and no specimen of '*rossi*' larva: so this was not a case of *culicifacies* being misdiagnosed as *rossi*.

We, however, obtained in March, during the detailed survey, one specimen.

To summarise, Watson and Strickland support one another in not finding the species from September to March which is presumptive evidence, in the absence of anything to the contrary, that at this season it is very rare.

Fry and McCombie Young in their turn both agree that the spring months, say March to May, are those of maximum prevalence.

Chalam and McCombie Young record that after the onset of the rains the species is scanty and the record of 31 caught in 7 months (including May, which is one of the optimum spring months) does not seriously invalidate the conclusion which we come to that the species is a spring-month species and not a cold weather or rains species.

The species like *maculatus* is usually found in pools in the beds of streams and is therefore liable to be washed away in the floods of the rains; however, it is unlike *maculatus* in that it does not become at all prevalent during the autumn and cold months. It has a much shorter season than *maculatus*, probably because it prefers pools of rain or river water while *maculatus* prefers spring water.

vagus.

Chalam and Young (1922) found this species very abundant during the rains. From May to November they captured 1,237 adults, representing about 50 per cent of their total catch. It was said to have completely disappeared during the cold and the dry season.

Watson (1924), in the cold months, caught only one out of a total catch of 2,345 mosquitoes, this lending support to the previous record.

Strickland (1924) reported the finding, during the months September to November, of 273 larvæ in a total catch of 1,730 (about 15 per cent), a fact also onsonant with the preceding records.

In the 1924 detailed survey we found in the half-year June to November, 228 breeding-places or 19·7 per cent of our total records, while from December to May we took 223 or 25 per cent, the latter finding lending support to the possibility that McCombie Young's report of *rossi* in the spring months had really reference to *vagus*, with which, of course, it has sometimes been confused.

Our quarterly catches of the species are shown in this table:—

Periods.	Number of breeding-places.
June to August	85
September to November	143
December to February	30
March to May	193

The smaller number found in the cold months confirms particularly Chalam, Young and Watson.

But the greatest number being found in the dry hot months does not confirm Young and Chalam who have held that the species completely disappears * during the dry season.

Our monthly record of breeding-places supports the general proposition that the cold months are inhibitory, while April to May are very favourable, the rains are inhibitory but with the cessation of the rains the species increases again slightly until the cold weather slump.

vagus being a pool breeder, usually in dirty water seems to be affected by the flush of the rains when the species undergoes a reduction in numbers. It is not found to any extent in the cold weather perhaps because the dirty pools have by then dried up.

Summary.

The conclusions which we have come to above regarding the seasonal prevalence of each species may be expressed in terms of four groups.

Group I.—The *winter anophelines;* comprising *gigas, fuliginosus, hyrcanus* (= *sinensis*) and to a less extent *maculatus* (note the absence of *culicifacies*).

Group II.—The *spring anophelines*; *maculatus, culicifacies* and *vagus* and to a less extent *barbirostris.*

Group III.—The *rains; barbirostris, kochi* and *tesselatus* and to some extent *hyrcanus* and *vagus*, and probably *umbrosus.*

Group IV.—The autumn group; in which is *umbrosus* and *hyrcanus* and to a less extent *kochi, tesselatus, fuliginosus, maculatus* and *vagus.*

Special mention must be made of *aconitus, funestus* and *jeyporiensis.* We found in our detailed survey that *aconitus* and *funestus* were very similar from the point of view of seasonal prevalence, so that they may be considered together. They appear to be prolific all the year round, decreasing somewhat in winter. *jeyporiensis* has not been found often and only during the rains and the autumn. Fry in the Bengal survey said 'The *Myzomyias* are so scarce that no accurate data of their seasonal variation can be given, though they seem more common in spring.

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* Probably a local manifestation.

Of the species *jamesi*, *karwari*, *leucosphyrus* and *aitkeni*, the records are too sparse to be of much use. *karwari*, however, is probably very like *maculatus*; and *jamesi* like *fuliginosus*.

The species *rossi* and *pallida* are probably not Assam species.

The percentage to total figures for some of the species in these groups during the seasons was as follows:—

GROUP I.—

	fuliginosus.	*hyrcanus.*
Spring	13·4	20
Summer (rains)	9·6	39·6
Autumn	18·4	43
Winter	28	47·2

GROUP II.—

	vagus.
Spring	46·2
Summer	20
Autumn	19·5
Winter	6·3

GROUP III.—

	barbirostris.	*kochi* or *tesselatus.*
Spring	10	1·4
Summer	21·3	4·7
Autumn	8·2	3
Winter	5	·7

GROUP IV.—Some of the species in this group are shown above.

(*h*) *The case-incidence.*

Appendix III C (h 1—18) and III C D (h×1).

The monthly case incidence on each of three estates, Khobang, Aenakhal and Phulcherra, has been charted in Appendix I, while the incidence on many other representative estates and that shown in the Dibrugarh Government Hospital records, has been charted in Appendix III C (h).

In the charts there is seen to be nearly always a maximum rise in August, September and October, after which till March a decline sets in. Then from this time the rate gradually rises till the maximum is reached. (Kamptigowalli, Gandicherra, Sagmootia or Dibrugarh may be taken as typical, but a comparatively high incidence in the cold months, December and January, does not seem to be uncommon.)

The correlated climatic conditions have been shown in Appendix I and it would appear that the case-incidence is roughly correlated with the temperature and the rainfall, a rise of both of which antedates the latter by about two months.

On the other hand, the Assam Administration Report of 1880-81 has it, that while the spring months had been very wet the fever season was marked by little sickness, in fact there was some sort of inversity between the two events.

McCombie Young (1922) talks about a spring rise dropping in monsoon months to rise again in the autumn as being typical of Assam generally, and of Lumding in particular; and again (1923) '..........a maximum in May ....coincides with the general wave of malarial incidence in the province,' from which it appears there is a decided difference in the records of Lumding and of Assam as a whole, on one hand, and the tea estates on the other.

Fry, speaking for Bengal, says that while autumn is the season of severe malaria, the records of the dispensaries prove that the season of new cases begins in July, and these apparently increase until a maximum of sickness is attained in October, in which morbidity is at its height. These conditions are what may be described as typical of the tea-garden malaria-incidence.

(*j*) *Mortality-index.*

The mortality rate has been charted in the case of a few estates only but do not, as was to have been expected with such small numbers, show any definite seasonal incidence except in one case where there seems to have been a sustained rise from August to December.

District mortality rates from fever as compiled by McCombie Young (1923 are given in the Appendix. They all show a definite peak at the opening of the rains. But these districts, it must be recalled, were 'kala-azar districts' and their malarial fever mortality rates may have been largely vitiated by this fact. In this connection it is interesting to note in Fry's reports on Bengal that there are two annual rises of mortality of which one, in April, may perhaps be due to kala-azar. The maximum fever mortality was in November, December and January. No correlation with rainfall could be made out.

Inter-correlations.

(*a*) (*b*) *The spleen-index of garden* plus *country-born children with the spleen-index of only the garden-born children.*

The spleen-index of the garden-born children was found to be about the same as that of the recruited *plus* the garden-born children. That is, in spite of the number of recruits yearly being considerable (in the year 1924-25 about 4 per cent of the total tea-garden population), each group rapidly tars the other with the same brush as itself. For large and quite small areas this is generally found to be the case, even in the monthly records. However, when one comes to observe the records of separate estates, it is not invariably true, consequently there is a limit to its practical application, and in taking the spleen-index of estates one must separate the garden-born sheep from the country-born lambs.

(The correlation of the spleen-index with the morbidity rate, etc., will be discussed later.)

(*c*) *the parasite-index with* (*f*) *the anopheline-index with* (*h*) *case-incidence.*

(The inter-correlations will be discussed below.)

(*f*) *The anopheline-index with* (*c*) *the parasite-index.*

Perhaps one may argue on the analogy of the periodicity of *filaria bancrofti* that anophelines or at least the carrier-species, and the malarial blood-parasites, become more prevalent at the same time.

(*h*) *The case-incidence with* (*a*) (*b*) *the spleen-index.*

We found, as stated above, that the spleen-index taken in two half-yearly periods did not vary much. In the second half, the index was only a little more than 2 per cent higher, and this was during the most highly malarious months. It seems remarkable that the epidemic season should not have been found to exert a greater effect on the spleen-rate. Probably, as Major Stewart suggests, in a lightly infected community the malaria epidemic season would influence the spleen-index to a greater extent.

(*h*)—*with* (*c*) *the parasite-index.*

The parasite-index which we found over quarterly periods was on all fours with the rise and fall of the epidemic season.

The main feature in the parasitage was the autumnal fall in the rate, this being followed by the winter decrease of malaria; and then in winter, spring and summer the gradual rise in the parasite-rate followed by corresponding rises in the malaria-rate to produce the annual epidemic.

(*h*)—*with* (*f*) (*i*) *the total anophelism.*

Anophelines are apparently less prevalent in the dry half of the year than in the rainy half, which includes the epidemic season: therefore the rains do not wash away the carrier anophelines.

During the quarterly periods (*see* Chart III A. h. f. (i) there is no apparent correlation between the data, probably because of the irregular covering of the numbers of the carrying species by non-carrying species.

(*h*)—*with* (*f*) (*ii*) *the anopheline-species.*

If we attempt to correlate the seasonal prevalence of the different anopheline species with the rise and fall of the case rate, we must say that our conclusions are influenced by a premiss that species which are known to be natural carriers (not 'laboratory' carriers) elsewhere may be carriers also in Assam, the assumption being justifiable, for there has never been any evidence to the contrary: and by another premiss that seasonal susceptibility of mosquitoes to malarial infection is the same for all the species of anophelines.

We will now consider the species in the groups given on page 58 and we show in the table chart subjoined the case-rate computed for the quarterly periods, against the corresponding anopheline species-index.

The highest case-incidence in autumn was correlated with *barbirostris*, the *kochi-tesselatus* group, and *funestus* and probably *umbrosus*, the *jeyporiensis* figures being too slight to be of any service.

The cold weather fever rate was associated with *umbrosus*, *hyrcanus*, *funestus*, (and again *jeyporiensis*).

The lowest rate in spring with *gigas*, *fuliginosus* and *hyrcanus*.

The rising incidence about the onset of the rains with *maculatus* (*karwari*) *culicifacies*, *vagus*, *funestus* and *aconitus*.

Now we know that of the species here mentioned *funestus*, *umbrosus*, *maculatus*, *culicifacies* and *aconitus* are serious natural carriers (*see*·Appendix III), and perhaps it would be unnecessary to look beyond them for any other causes of the malaria in Assam; perhaps they only and their relative seasonal-prevalence may explain the high malaria-rate in the autumn months, the decline, the stagnation and the onset of the epidemic.

Of these malaria carriers special mention must be made of *funestus* and its associate *aconitus*. The number of its 'breeding-places' found in comparison with other species is normally small but it is a highly infective mosquito, prevalent all the year round, and its domestic habits make it all the more dangerous. All previous observers have concluded that it is a species which is a very important factor in Assam tea-garden malaria. *culicifacies* and *maculatus* are both probably important but show an interesting distinction. Both breed in pools in the bed of sandy rivers and nullahs but whereas *maculatus* is found in the cold months, not so *culicifacies*. At this season the low fever-rate is no doubt partly due to that reason.

maculatus was incriminated by Watson as probably the cause of much malaria at Doom Dooma and by McCombie Young at Pasighat, while *culicifacies* was blamed by McCombie Young at Lumding.

We perhaps may rule out of our condemnation *aitkenii*, *gigas*, *jamesii*, *karwari*, *kochi-tesselatus* and *leucosphyrus*, on the ground of their relative paucity of numbers and because there is no evidence that they are malaria-carriers. *karwari*, however, in view of its association with *maculatus*, might be investigated further, and is probably commoner than is supposed.

barbirostris calls for special consideration, as beside its enormous prevalence at the rise of the annual epidemic, it has been proved susceptible to malarial infection. It is possible that this species has something to do with the annual epidemic and it should certainly be enquired into.

barbirostris during the preliminary survey, was found breeding ca. 400 times, as compared with *funestus* and *aconitus* ca. 136 times, while in the detailed survey the relative figures were about 250 to 50. With this relative preponderance of *barbirostris* it need not have a high infective-rate to do as much damage as *funestus*, but of course other factors, such as 'domesticity,' have to be taken into account.

fuliginosus also needs special consideration; its enormous winter prevalence, as Fry has emphasised in Bengal, is correlated with the lowest case-incidence during the year; so that in spite of its undoubted sporadic infectivity it can have but little epidemic significance and might be disregarded.

hyrcanus like *fuliginosus* is specially prevalent in the winter and associated with the fever-rate when it is lowest, while in autumn it reaches its maximum prevalence while the malarial-rate is declining. The evidence is therefore against it being a factor of any importance.

jeyporiensis, of the susceptibility of which to infection there seems to be little evidence,* should, in view of the fact that it is a *Myzomyia* (*sensu restricto*), be further investigated. However, it appears to be very rare in Assam and in any case would be of only local significance.

The case-incidence described by McCombie Young as having two peaks, one in April and May and one in the autumn (for example at Lumding) is obviously due to peculiarly local conditions of mosquito production; and *culicifacies* and *maculatus*, as he infers, must be chiefly involved. These mosquitoes are 'dry' weather breeders and when the rains come they are washed away. Other peculiarly local types of case incidence, differing from Assam as a whole, would probably always be found due to correlated atypical mosquito production, each type of case-rate depending on particular species of mosquito and their breeding-places.

(*j*) *The mortality-index with* (*h*) the *case-incidence.*

Unfortunately the official figures for the whole of Assam are not available, as they would probably throw some light on the inter-relations of these indices.

Fry, in Bengal, found that the sickness-rate antedated the mortality-rate by about two months.

The evidence gathered in our year's survey is too slight to be of any value but there are indications in the chart for Khobang Tea Estate (Appendix I) that Fry's experience in Bengal is the rule also on the tea-estates.

One should, then, following the trend of the evidence, take special precautions and adopt special treatment during the cold months when the mortality rate may be expected to be highest.

SECTION B.

The annual incidence of malaria.

The only data under this heading reported on here are shown in Appendix III E (*h*) as Charts (1—5), from which it appears that the case-rate cannot be definitely correlated with any annual climatic condition.

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* Recently annotated by Covell from *Ind. J. Med. Res. Memoirs* No. 7, July, 1927.

Fry, it may be noticed in this section, says that the year 1908, in which there was a marked rise in food prices in Bengal, was remarkable for its comparatively low mortality, and thinks that when prices go up the people sell only their surplus and profit by the high prices obtained, an example of what is now called 'bonification'.

However, it may also be remarked that this year was associated with a very low December temperature, a circumstance which in itself might have accounted for the low mortality.

Summary.

(1) Seasonal factors do not influence the spleen-index in Assam tea gardens to any appreciable extent.

(2) The parasite-rate varies at the different seasons, the variation not being correlated with meteorological conditions.

(3) The hæmoglobin, like the spleen-index, does not vary much between different seasons.

(4) Anophelines are more plentiful during the rainy-half of the year and of this period the months after the active monsoon are most productive of them.

(5) The different species in relation to the various seasons have been carefully considered, and their classification into four corresponding groups definitely established.

(6) The case-incidence in the gardens differs from that of Assam as a whole as stated by McCombie Young, and forms a regular and symmetrical peak about September or October.

(7) There was but little evidence to show the season of greatest malaria mortality on the gardens.

(8) The spleen-indices of the garden-born children and of the country-recruited children reach the same level in a short time. It is, however, necessary on estates to rely upon the index of the garden-born children only, as an index of endemicity.

(9) It is suggested that on the analogy of the periodicity of *Filaria bancrofti*, the malaria-parasites and the carrier anopheline-species are more prevalent at one and the same time.

(10) The epidemic season does not influence the spleen-index to any extent.

(11) The parasite-index appears to be correlated with the epidemic season.

(12) The correlation between the annual epidemic and the prevalence of the various species of anophelines in terms of the seasonal groups which were established above is discussed. The common malaria carriers elsewhere may be incriminated in

Assam, *barbirostris* may cause some trouble, but *hyrcanus* and *fuliginosus* can be ruled out.

(13) The mortality from malaria probably postdates the sickness-rate by about two months.

(14) The epidemics in various years have not been correlated with any meteorological conditions.

PART IV.

THE RESPONSIBILITY FOR THE ENDEMICITY OF MALARIA OF CERTAIN ELEMENTS IN THE TERRAIN.

By

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and

K. L. CHOWDHURY, M.B., D.P.H.

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PREFACE.

In Part IV of the Original Report was submitted a short account of the material collected by the survey on each garden, the accounts being given in regional groups, at the end of each of these a 'discussion' being joined on any points which appeared to stand out as of special interest. Then these 'group' conclusions were considered altogether, and further conclusions come to which seemed to hold good for each district; and finally so also for the two great valleys.

From this Report the detailed account of each estate with the appendices have been deleted, but the data will be distributed by stencilscript to the local interests, or filed in the office of the Indian Tea Association, Calcutta, for reference if required.

In what remains there may be various errors, but the import of even the most serious is eliminated in an extended survey such as ours was. Even false conclusions may have been drawn, though here again fallacies tend to be submerged in such an extended survey, i.e., if too much stress is not laid on apparently exceptional occurrences. One may draw false conclusions on one garden one time but not on all gardens all the time. However, we have thought it wisest to base our opinions primarily on the places of which we ourselves were personally cognisant, treating the evidence of our field-assistants as confirmatory of our own hypotheses.

B. THE ASSAM VALLEY.

BA. LAKHIMPUR DISTRICT.

Discussions on Groups.

Ba 1. Gardens 1 to 6 in the Talap group, Khobang, Liana, Talap, Dangri, Tiphuk, and Dholla.

In general, it may be said that the lines of these estates are highly malarious if they lie near to a perennially-running stream or to a swamp, but if, as on Khobang, there is an absence of such conditions, the malaria-index is not high, while Tiphuk indicates that it is the streams which are malariagenic, for on this estate there is a comparative absence of swamp.

Ba 2. Garden 1 in the Daisa-nadi area. Daisajan.

One may conclude that most of the malaria in its malarious lines is derived from the jan.

Ba 3. Gardens 1 to 7 in the Hukanguri area. Hukanguri, Hillika, Deamoolie, Dhaimukhia, Beesakopie, Sookerating, and Tara.

This group goes to prove that the nearer lines are to perennial water the more malarious they are. Hulas, which dry up in the cold weather after the rains are over, are not so dangerous. Where the bed of a river like the Dibru has a number of small streamlets draining away its swamps, there is more malaria than there is where there is but little swamp and few streamlets. One set of lines on Tara would support an hypothesis that jungle is particularly dangerous, though the jungle mosquito *umbrosus* was not caught in the vicinity.

Ba 4. Garden 1 in the Hapjan group. Hapjan.

This estate is situated on low banks but the spleen-index is nevertheless very high. There is here probably a minimal amount of seepage along the lower contour of the banks and *maculatus* would therefore not be favoured. On the other hand the proximity of the lines to a big grassy jan, just as in the case of the Dibru river jan, seems to be the main exciting cause for the high malaria-rate.

Ba 5. Gardens 1 to 6 in the Dinjan area. Panitola, Nokhroy, Woodbine, Rangagora, Limbuguri, and Kutchujan.

Looking broadly at the data collected in this area there is really not a great deal of difference between the different estates. One of us (C. S.) in 1923 obtained the maximum spleen-index both in the Hospital lines, Kamptigowalli, and in the Jorai-Norhat lines of the Panitolla home division. This similar hyperendemic index all over the group is associated in varying degrees with proximity to jungle and thatch, jans with and without perennial water, khet land and the Dibru Forest

Reserve. It is therefore impossible to assign definitely the part played in the malariousness of the place to any one element of the terrain.

Ba 6. Gardens 1 to 8 in the Dibrugarh-Balijan group. Barbaree, Maijan, Rajgarh, Greenwood, Nagadhuli, Hazlebank, Nahor and Balijan.

The estates in this group indicate that land on the pathar level when cultivated is comparatively harmless while swampy land is rather harmful. The gardens near the Maijan, which does not carry perennial water and is cultivated with paddy, showed a higher spleen-index, (in the neighbourhood of 25 per cent), and this can be only explained on the assumption that swamp lies in and drains away from the Maijan for a sufficiently long period during the rains to develop a harmful situation.

The gardens nearer the Dibru River, as Dr. Murphy has remarked (when forwarding certain indices for our information), are definitely much more seriously infected (Nagadhuli and Balijan). Its valley is a mass of swampy bhil in grass, jungle and a few patches of paddy.

A small stream like the Dinjan with perennial water on the pathar level develops about the same low spleen-index as the considerable valley of the Maijan.

Ba 7. Gardens 1 and 2 south of Dibrugarh. Biheating and Barbarua.

In this group it appears that lines adjoining the bank on which there is no natural perennial water and situated on the pathar level are comparatively healthy, while a tank on the bank is associated with a much higher spleen-index, though not a serious one. Perhaps the tank does not develop so much ill-health as the seepage swamps, which perhaps breed *maculatus* at the foot of the bank.*

Ba 8. Gardens 1 to 5 near the Tingrainadi. Tingri, Keyhung, Dirial, Hoogrijan, and Rajah Ali.

The only explanation that can be offered for the comparative good health of Rajah Ali and Dirial is that their lines do not lie close to the Tingraijan as in the case of the other estates in this part of the district. Jungle when not with perennial swamp is also proved not to be of great danger.

Ba 9. Garden 1 in the region of the Disang. Namrup.

This estate is instructive as showing the comparative benefit of having lines on the so-called pathar level, as apposed to a site on a tea bank.

Ba 10. Gardens, etc., 1 to 4 in the Burhi-Dihing group. Makum, Namdang, The Railway Sawmills, and Digboi.

This is a very malarious group. The hulas on the great tea bank at Margherita do not all carry perennial water, but when they do, in their grassy-edged streams many *aconitus* and *funestus* were caught.

Another source of infection is probably the bed of the Burhi-Dihing and its tributary the Makum Pani, where *culicifacies* was found in pools left by the receding stream.

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* Since writing the above, Dr. Tudor Williams has informed me that the bulk of the coolies on Barbarua do not live near the tank but elsewhere.

maculatus might be expected in seepage swamps anywhere at the foot of the great banks of porous soil which constitute the tea land but this species was not actually found.

It is instructive to note that the sawmills at Margherita showed not such a high splenic-index. Except for the Dihing as a source of infection, there was so much *jabra* and jungle in the vicinity that no anopheline had much chance to 'breed'.

Ba 11. Gardens 1 to 3 in North Lakhimpur. Dhoolahat, Silonabari, and Tonijan.

The discovery of *maculatus* on Tonijan probably furnishes a clue to the malariology of this region. The streams coming down from the hills leave pools of clear water with a bed of silt, an ideal breeding-place for this mosquito, and perhaps *culicifacies*.

CONCLUSIONS.

Regarding Malaria in Lakhimpur District.

This district is particularly interesting as it is fragmented by many great bhils or jans in various stages of decay which have been left by the great Brahmaputra. They dominate the local scenery, bounded as they usually are by abrupt banks on which the tea estates are planted.

Various groups of these gardens which have been examined have enabled us to draw some very definite conclusions regarding a few of the factors conducive to malaria.

Estates are invariably malarious if near perennial streams or swamps, and where there is no swamp or perennial stream the malaria-rate is not high. Tiphuk Tea Estate proves that streams *per se* are malariagenic, for here there is no swamp or other possible source of malaria. Almost invariably we found that the nearer lines are to perennial water the more unhealthy they are. The comparatively good health of Rajah Ali and Dirial must be due to the fact that their lines are nowhere near the Tingraijan. The so-called bhils or jans in this District (especially, it was seen, in the Doom Dooma area) are very often filled with unfordable swamp showing up, here and there, lenticles of higher ground and overgrown with every species of jungle and swamp vegetation that finds the particular conditions to suit it. In these swamps, streamlets loom up and take shape marking out courses for themselves and eventually unite to form considerable streams: in these streamlets *funestus* and *aconitus* are the common and dangerous malaria carriers, while Sir Malcolm Watson at Doom Dooma found numerous *maculatus* living in the seepage swamps or springs at the foot of the great bounding banks above which the tea is grown.

Or, where the process of extinction of the old river beds has progressed to a further stage, perhaps it is all planted up with paddy, e.g., the Maijan, and carries no perennial water, and may be only acting as a temporary sump for the storm-water of a very small area.

The ill-health of lines situated along these bhils is a measure of the number of perennial streams and swamps in them. Dr. Murphy in a letter to one of us noted that the nearer estates are to the Dibru River with its grassy swamps, jungle and labyrinth of streams the more unhealthy they are, while Sir Malcolm Watson in his report on malaria at Doom Dooma (1924) notes the same thing. An estate like Sookerating is less malarious because there the bed of the bhil is generally higher and dryer and there are fewer streamlets and swamps. Paddy cannot be grown in deep swamp, so generally speaking a paddy-planted bhil or jan is healthier than a jungly swampy one.

When one comes to bhils like the Maijan which is largely planted with paddy and carries no perennial water, acting merely as a sump for the immediate rainfall, one finds the health comparatively good, the spleen-index being about 25 per cent. When comparing the harmfulness of streams and bhils on the pathar level, it was interesting to note that a small active stream near to the Maijan seemed to be responsible for about the same spleen-index as the very much greater extent of the Maijan itself. The same sort of spleen-index may be expected in the neighbourhood of any swampy unplantable ground, (e.g., Borbarrie Tea Estate near Dibrugarh).

An important observation was made at Hapjan. Here the lines lie under the influence of one of these bad grassy swampy jans and have a maximal spleen-index. The notorious gardens at Doom Dooma have no worse, in fact slightly better. Now the Doom Dooma gardens mostly lie on very high banks at the foot of which, at the fall of the year, seepage swamps and with them *maculatus* make their appearance, as Watson has reported. Hapjan, on the other hand, lies on very low banks between its jans and there can be little or no seepage swamps anywhere, with their consequent *maculatus*. The conclusion to be drawn is that in the Doom Dooma gardens just as at Hapjan nearly all the trouble is probably coming from the great bhils and the influence of the seepage swamps at their margin is negligible. We would not for a moment attempt to urge that the *maculatus* factor should be disregarded and nothing done to eradicate it, but we think that the above truly represents the relative importance of it and the bhil factor.

Estates planted on the pathar level and surrounded by rice cultivation were found to be comparatively healthy, e.g., Biheating with a spleen-index of about 6 per cent, while immediately adjacent on the banks the spleen-index was higher. In the pathar gardens observed there was no perennial water near, but they were exposed to any *maculatus* which might have been breeding in swamps at the foot of neighbouring banks.

Tanks. In one garden, Barbarua, planted on higher ground above the pathar level, the only source of a 25 per cent spleen-index which could be ascertained was a large tank round which some lines were grouped in the centre of the garden. We, however, feel rather doubtful whether there may not have been some other reason for this spleen-index, such, for instance, as a seepage swamp at the foot of the tea bank.

Hulas. Generally speaking, the district is so scarred by jans that there is very little room or need for hulas as drainage channels, but a few were observed and notes made concerning them.

On one estate some lines near some hulas which only ran water for a short time in the year were very much healthier than others situated alongside a big jan. The spleen-index near the hulas was about 50 per cent.

On an estate near Margherita there were many hulas on the tea bank and, some of them ran perennial water. Many of them contained *funestus* and *aconitus*, but the lines near them with 100 per cent spleen-index were likewise near another possible focus of infection, so that the fractional influence of the hulas in this case could not be determined.

Active Rivers. Both Sir Malcolm Watson and ourselves have found *maculatus* in silted pools of the Brahmaputra, but we have had no observations as to any effect on health such breeding-places have had. Lt.-Col. McCombie Young at Pasighat found numerous *maculatus* in the pools left among the shingle of the Dihang River.

In North Lakhimpur we found *maculatus* breeding in the silted streams of some estates, while in pools in the bed of the great Dihing River at Margherita and its tributary the Makum Pani we obtained specimens of *culicifacies.**

The silted or shingly beds of all active rivers must be suspected of carrying *maculatus* and *culicifacies*, the former particularly where there are springs of water in the bed.

Jungle. Many very unhealthy estates come into close relationship with jungle, but there is sufficient to account for their ill-health from the fact of proximity to perennial streams, or swamps, without incriminating the jungle. In the case of the estates along the Dibru River it is probable, nevertheless, that some of their ill-health is coming from the Dibru Reserved Forest, breeding *umbrosus*. On the other hand some comparatively healthy estates were observed which were surrounded by forest, but in them there was no perennial water, and hulas were markedly absent.

On one estate we observed a line surrounded closely by forest with a spleen-index of about 100 per cent and no explanation of any sort for this could be found in the time at our disposal.

Bb. SIBSAGAR DISTRICT.

Discussions on groups.

Bb 1. Garden 1 in the Sibsagar Sub-division. Borhat.

The malaria sickness rate here appears not to be a serious matter. It may be more than a coincidence that the comparatively healthy Tin lines lie in relation to jungly hulas.

Bb 2. Gardens 1 and 2 in the Janti River area. Boisahabi and Seleng.

In this group the splenic-index was moderately high, except where, it appears, the lines were situated in closer relationship to the dhan-khet land, the index here being lower.

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* Compare the considerable silted stream at Lumding in which *maculatus* and *culicifacies* are commonly found and are probably responsible for the residual spleen-index in the station of about 30 per cent, now that most of the endemic malaria has been eradicated by anti-malarial measures.

Bb 3. Gardens 1 and 2 in the Cinnamara circle. Cinnamara, and Dakiajuli.

The group shows a moderately high endemic index without much variation. On Cinnamara, the garden is bounded by some considerable streams of perennial water, and the same is the case with Dakiajuli.

Bb 4. Gardens 1 to 7 in the Jorhat Sub-division north of the Dholi River. Panbari, Kataribari, Mariani, Dholi, Kharikatia, Sycotta and Barsycotta.

An analysis of the findings on the gardens of this group leads to some interesting conclusions. Panbari lines and some lines on other gardens are comparatively free from malaria and they are placed on very low banks practically surrounded by dhan-khet land on the pathar level and they are near to no permanent streams. Nevertheless, the lines lie in some relation to shallow hulas of the usual type which run with clear water in the rains, which proves the comparative innocuousness of these hulas.

Then comes Dholi Estate which is exactly the same with the addition of a perennial stream in the background and it has a higher index.

Then come other estates with spleen-indices of from 30 to 50 per cent, all more or less near to perennial streams.

One or two of the estates, e.g., Sycotta, have no marked hulas, only drained depressions in the tea, and their spleen-indices vary from 20 to 50, according to the distance from a perennial stream.

Bb 5. Gardens 1 to 5 south of the Dholi River in the Jorhat Sub-district. Bokahola, Rangajan, Helikha, Nagadhuli and Halijuri.

Bokahola and Rangajan prove that the proximity of lines to a perennial grassy stream is more dangerous than are any hulas but that does not necessarily lead to a high spleen-index: theirs varied from 14 to 26 per cent. Nagadhuli was in much the same condition, except that there was a very long hula in addition and it had a spleen-index of about 50.

It was observed in this region that the fact of lines being near to undrained jungle did not lead to a higher spleen-index.

Bb 6. Gardens 1 to 3 in the Jorhat Sub-district between the Kasojan and Akyajan. Titabar, Matijan and Leatakujan.

This is a very instructive group of three rather small estates. In none of them is hula sculpturing of the surface of the bank on which the gardens are planted a marked feature, so hula influence is probably negligible. Then the value of the spleen-index is seen to be beautifully correlated with the distance of the lines from perennial streams. An interesting point was observed in this region that a line backed by heavy forest does not necessarily have a high spleen-index; (18 per cent) and it appeared, moreover, in this instance that a belt of jungle interposed between a permanent breeding-place and human habitations was able to act as some protection to them.

Bb 7. Garden 1 on the Naga Hills border of the District. Hunwal.

One would expect the spleen-indices of lines, which lie so close to a perennial stream such as the Chuakhati, to be higher.

Bb 8. The Kakadanga River Group. Mohima, Daflating and Chunghee.

Daflating and Chunghee are both rather similarly situated in relation to big streams, but their respective spleen-indices are rather wide apart. The reason for this is not clear.

It is interesting to note that the former estate being planted on a higher bank than the latter has a comparatively lower spleen-index. If there were much seepage from the bank breeding *maculatus*, one would expect a much higher spleen-index.

Bb 9. Gardens 1 and 2 in the Golaghat Sub-district. Jamjuri and Dayang.

The lines of these estates have a fairly high spleen-index of about 40 to 60 per cent. This may be due either to the Dayang River which, however, is some distance (one to two miles) away, or to the few small perennial streamlets that exist near at hand, or to the network of old river beds and bhils and hulas which fragment the gardens. The latter, however, do not carry permanent water, and to which of these elements in the terrain the malaria is due or in what proportion was not determined.

CONCLUSIONS.

Regarding Malaria in Sibsagar District.

In this district some quite definite facts were elucidated from a consideration of the findings in the different groups of gardens.

Those situated in close relationship to dhan-khet on the pathar level were always healthier than others, and never had a higher spleen-index than about 20 per cent, but none of the lines involved were near perennial streams. In the dhan-khets the common anophelines were *fuliginosus*, *barbirostris* and *sinensis*.

In some of the estates away from any possible influence of perennial streams and with lines showing such low spleen-indices as mentioned above, the tea was planted on very low banks, sculptured by shallow hulas in which run a little water during the rains, while on the other hand in some, as at Daflating, the tea bank was very high and abrupt. The height of the bank then seemed to make no appreciable difference to the incidence of malaria, an additional proof that *maculatus* breeding in spring water seeping through such banks is of no great importance.

One of these estates with a spleen-index of under 15 per cent was about a mile from the Dholinadi, a demonstration of the amount of harm that can be expected from a perennial stream at this distance.

As soon as an estate otherwise similar to those above mentioned is placed near a perennial stream, its spleen-index is raised, e.g., Dholi Tea Estate. Spleen-indices of from 20 to 50 per cent are found closely correlated with the distance away from such a stream. In this district this correlation is particularly prettily shown because many of the gardens are scarcely marked by a hula, storm water being carried off in the slightest of depressions on the surface of the land.

On the other hand apparently certain lines of Bokahola, Rangajan and Hunwal have a spleen-index considerably less than their proximity to perennial streams would seem to warrant, and such exceptional occurrences really should be further studied, as they might be of practical importance.

Hulas. Those estates with a few lowly graded shallow hulas have a low spleen-index and Daflating with higher graded hulas on an island of land arising out of the pathar has likewise but a small spleen-index.

On the other hand a hula containing a well-marked nullah which does not, we understand, contain perennial water (Nagadhuli) seems capable of producing a 50 per cent spleen-index. It stands to reason that watercourses vary in every degree from those in which there is only water during rainfall to those in which water is perennially running, the chief factor probably being the amount of subsoil water taken up and the time this takes to flow away. A nullah carrying water then only a little short of perennially, may be expected to cause nearly as much harm as a perennial stream.

There is one type of hula in which the bed has been scooped out sufficiently widely to be swampy or planted with paddy during the rains, and lines near to such hulas show a spleen-index of about 50 per cent.

Bhils are not common in the tea area of Sibsagar District, but estates which are fragmented by them and are also in close relationship to perennial streams show rather a higher index than would probably be accounted for by the streams alone.

Jungle and forest lines on estates near to undrained jungle did not have a higher spleen-index than one would expect if the situation were opened up. In fact, in one instance the spleen-index of a line backed by heavy forest was only 10 per cent.

In another instance a belt of jungle interposed between a permanent breeding-place and some lines seemed to act as an efficient protection to them.

Bc. Darrang District.

Discussions on groups.

Bc 1. Gardens 1 to 9 on the Redbank. Hanchara, Adabari, Hathibari, Paniputa, Sonajuli, Namgaon, Nahorrani, Rupajooli and Kacharigaon.

A perusal of the data shows that there is a general high endemic rate on all these Redbank gardens, a fact which was brought to the notice of one of us (C. S.) by Dr. Forsyth immediately on the arrival of the survey party in these parts.

It is interesting to note, however, that those gardens situated near the pathar level were neither more nor less malarial on the average than those at a distance from it. Another point is that nearly every line investigated was away from any possible influence of jungle.

Presumably, therefore, the open hulas which sculpture the land everywhere, provide the sources of the malaria, and they were found to harbour the bad species *funestus* and *aconitus* as well as larvæ of either *maculatus* or *karwari* in numbers. Other less or non-dangerous species on the Redbank hulas were *vagus*, *sinensis*, *barbirostris*, *fuliginosus* and *kochi*.

The hulas and bhils on the Redbank are of every type, from steep narrow valleyettes exhibiting all active conditions (hulas) even to back flooding of their lower ends, to broad, shallow tracts which obviously represent moribund or dead river beds (bhils). No correlation could be elucidated between these various physiographical details and the slight variation in the general spleen-rate, though it seemed very remarkable that in the moribund bhils *fuliginosus*, *vagus* and other slight malaria carriers were the usual species while in the *hulas* the malaria carriers were commoner.

Another point which may be mentioned was the occasional connection of jungle with the malaria variation in different lines. Three lines the 'Porja' and 'Hospital' on Kacharigaon, and 'Tin' on Nahorrani, were all in close relation to jungle, but their malaria-rates showed no important variation from the average, which, of course, only proves that probably the jungle was equally malarious with the opened-up land. Here we found the notorious jungle malaria carrier *Anopheles umbrosus* as well as *barbirostris* and *aitkeni*. The remarks made by Dr. Forsyth, in his contribution in Part II, should be specially perused in this connection.

In conclusion, it may be said that a special survey of the Redbank gardens from the point of view of variations of malaria incidence in relation to variations in the physiographical development of the land surface would be both interesting and important.

Bc 1 and 2. Gardens showing transitional conditions between the Redbank and the pathar level. Katalguri.

The lines No. 7 of this estate were visited and the children showed an index of 60 per cent. The conditions were intermediate in type between those on the Redbank and on the pathar level, and very definite hulas were present. The spleen-index, it will be noted, approximated to those usual on the former.

Bc 2. Gardens 1 and 2 on the pathar level. Katalguri and Bindukuri.

Dr. Bentley's spleen-indices for gardens in this group some years back were as follows :—

Katalguri	9 per cent
Bindukuri	8 per cent

So that it appears that a higher endemic index has now been reached than formerly.

A possible reason for the increase on Katalguri is that the lines as examined by Dr. Bentley were perhaps not where they now are. The Burra lines, as examined by one of us (C. S.), were not a great distance from the Redbank and possibly suffered from this circumstance.

The mosquitoes found here on the neighbouring pathar were *barbirostris, fuliginosus* and *sinensis* and on similar rice land elsewhere in Darrang District we never obtained any malaria carriers. Dr. Bentley's figures are probably more truly representative of the pathar level than ours.*

Bc 3. Gardens 1 to 3. Panarihat, Dimakuchi and Kharkaria.

Nothing of value can be deduced from these observations.

Bc 4. Localities 1 to 3 in the Balipara Frontier Tract.

(1) *Chardwar.*

This is the Political Officer's headquarters. His bungalow was formerly so unhealthy that it had to be abandoned, but Captain Nevill had the surrounding jungle cleared for a distance of about a quarter of a mile and the site has since been comparatively healthy.

It lies on what may be called the pathar level of the Bhareli River and some of the land previously under jungle which was cleared was the so-called 'kurkarnimati,' the humps and hollows of which would naturally be productive of numerous anophelines. The pools of water so encouraged, however, soon dry up after the rains.

(2) *Daphla changs near to Lokra.*

These changs were built on dry ground cleared and reclaimed from the jungle. Though quite buried in the jungle, they were not very far from poor fields of rice cultivated by the Daphla folk.

These people were terribly disease-stricken (Fig. 5) and showed a spleen-index in 11 children of 100 per cent, in each case the spleen being near the *umbilicus*.

In the jungle roundabout, which lay on 'kurkarnimati' at any rate in some patches, *umbrosus* larvæ were found in some numbers, with *barbirostris* (*see* Strickland 1923, corr.), and may be presumed to have been the cause of the high malaria rate.

(3) *Lokra.*

Captain Wright, I.A., O.C., the 2nd Assam Rifles, at Lokra, has been good enough to send one of us (C. S.) some information with a plan which is not here shown † regarding the health of his command. This is reported to be good, at any rate much better than at Kohima, Ajal, Manipur, or Sadiya where the other battalions are, nevertheless there is an increase of hospital admissions for fever after return of the outposts on April 15th and the malaria season then lasts till September. The spleen-index of the children in the camp was given as 5 per cent (number not stated). This camp is interesting because it is adjacent to the jungle in which the species *umbrosus* was first found and it is important to note that men and children appear not to suffer much in health, probably because the clearing is wide enough to obviate any harm. If all the jungle hereabouts were cleared, the site of the camp would be considered to be at pathar level.

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* Since this was written, I have come to the conclusion that our figures are not abnormal—(C. S.).

† Filed for reference at the office of the I. T. A., Calcutta.

The differences evinced between these places resolve themselves mainly into a matter of the amount of jungle here and there. They all lie on the so-called pathar level which seems to be specially subject to the formation of 'kurkarnimati' with its consequent breeding-places for mosquitoes.

The Daphlas closely surrounded by jungle are very highly infected, while the station at Chardwar after being cleared, and the camp at Lokra have enjoyed fairly good health. Chardwar is near a big stream and the relation of this to anophelines was not ascertained.

FIG. 5.—These people are terribly disease-stricken.

In connection with our visit to the Balipara Frontier Tract, one of us (C. S.) wishes to express his best thanks to the Political Officer, Captain Nevill, I.P., for his help received.

CONCLUSIONS.

Regarding Malaria in Darrang District.

It is not right perhaps to claim any conclusions drawn from but one of the three divisions of the district as applicable to the whole. Only the central division was thoroughly investigated and it cannot even be said whether this is, or is not, typical of the whole.

One most important fact was elucidated here, that jungle is potentially dangerous. The jungle species *umbrosus* was discovered in pools and in a bhil, under the jungle, and evidences of its depredations were obtained. The fact that certain cooly lines situated at the edge of jungle were approximately as malarious as those in a corresponding situation where there was no jungle proves that the two conditions are much the same from a malaria point of view.

It was also seen on the pathar level how clearing the jungle for some distance round human habitations was a very effective anti-malarial method, as Watson so clearly showed in Malaya. The pathar land is very subject to the formation of what is called phonetically 'kurkarnimati,' among the excrescences of which pools of water are very apt to lie and if under jungle 'breed' *umbrosus*.

The tea estates in this part of the district are well known to have a very different malaria rate on the Redbank and on the pathar level. The spleen-indices which one of us (C. S.) took amply confirmed this view—those obtained on the Redbank and even down below when not far from the bank were universally high while on the pathar the garden-born children showed an index of round 25 per cent.

In the hulas which sculpture the surface of the Redbank everywhere were always to be found *funestus* and *aconitus*, and larvæ which might have been either *karwari* or *maculatus*; while on the pathar-level no malaria carriers were found.

It was not ascertained to what extent the Redbank is supplied with perennial streams, but probably most of the hulas only run during the rains and in the autumn.

The gardens at the edge of the great bank to the east overlooking the Barelli stream were certainly no more malarious than those on the bank away from its edge. This proves that mosquitoes (usually *maculatus*) breeding at the foot of the khud, in the spring pools there, are no more dangerous than those breeding on top of the bank. The hulas on the bank are very often cultivated with small fields of paddy and the spill water usually collected into a central stream, in which *funestus* is very prolific. In hulas under grass and swampy dirty water it was not so easy to find pathogenic mosquitoes as in those under paddy. Similarly in a *bhil* of stagnant water malaria-carrying mosquitoes were not found.

Bd. Nowgong District.

Discussions on group.

Bd. 1. Gardens 1 to 10. Rangimatti, New Salana Tea Estate, Lengteng Tea Estate, Jangobabusti, Kellyden, Meesa, Amluckie, Borghat, Sikini and Sagmootia.

The estates which were examined in Nowgong District are here not grouped, as it seems difficult to find a formula to warrant such a procedure.

They all lie between the foot of the Naga Hills and the busti khet land of the Kalang loop of the Brahmaputra.

Generally speaking the region is very involved in jungle, interspersed everywhere with patches of grass land, permanent swamp, wet paddy, or ***aush-dhan.*** The natural features which present themselves are not bold enough to enable one to connect them with the relative amount of malaria thereabouts.

This area has been in the past very seriously affected with kala-azar, but Dr. Dodds-Price has now got this disease well under control and on no estate can it be considered to have had any influence on the spleen-indices which were obtained (*see* Dodds-Price and Strickland, 1925). The gardens near the hills showed a very high spleen-index. If one may venture an opinion regarding the malaria on Meesa and Amluckie on the evidence so far adduced, it is that the hulas cause their high splenic-indices, while in places like Rangimatti and Jengdoba the expansive permanent swamp is probably very dangerous. At Amluckie the hulas are particularly shallow and near the pathar-level the spleen-index was only moderately high.

CONCLUSIONS.

Regarding Malaria in the Nowgong District.

There are, comparatively speaking, not many estates in Nowgong District, but a good sample was visited during the preliminary survey and thoroughly examined.

The natural features of these estates are more or less jigsawed into each picture and are not, therefore, as said above, bold enough to warrant one in connecting any of them with the incidence of malaria.

It is important to note, however, that in a bhil here in one of the reserved forests *umbrosus* was found and as there is a good deal of jungle about the tea gardens probably it is causing some mischief.

A characteristic feature was the enormous numbers of ***barbirostris*** caught in the district, as one would expect in such a jungly region. At the same time in even the most jungly of gardens there was never any difficulty in obtaining ***funestus*** from the human habitations, though very few of their larval habitats were found.

The spleen-indices of gardens like Sikini and Sagmootia which lie up against the hills and not in close relation to swamp and are marked by hulas which do not carry permanent water are very high. Estates like Amluckie that have their lines near to the pathar-level though also near to shallow hulas have a lower index, while those like Rangimatti which are mixed up with permanent hill-foot swamp partly converted to paddy cultivation are very malarious, and in the latter ***funestus*** is very prolific.

MALARIA IN THE ASSAM VALLEY.

Active rivers. A great river like the Brahmaputra is apparently not associated with any serious malaria in those living near to it. Its bed and banks are composed of a fine silt on which at the cessation of the rains as the river goes down pools for a time persist, ***maculatus*** being found in them (Fig. 6).

A river of the second magnitude, e.g., the Dihang at Pasighat (McCombie Young) which is strewn with boulders, is a favourite breeding-place for ***maculatus*** and responsible for a high malaria rate : and on the great Dihing at Margherita in pools lying on its bed we obtained *culicifacies.*

In rivers of the third magnitude (Fig. 7), e.g., the Makum Pani tributary to the Dihang, or the Horu Langpher at Lumding, *culicifacies* and *maculatus* may be found in pools left by the receding floods, and in the case of the last place the amount of malaria caused by such a site has been measured and is about 30 per cent. This estimate was made possible by the fact that at least most other possible sources of infection have been eliminated by thorough anti-malarial operations. The malarial incidence for which these rivers are responsible is therefore variable and is probably dependent on the recession of the waters. A river filled with boulders provides more breeding-places than one filled with silt, other things being equal and is therefore more dangerous. Also other things being equal, e.g., the character of the texture of the bed of the river, the smaller the stream the more malarious it probably is.

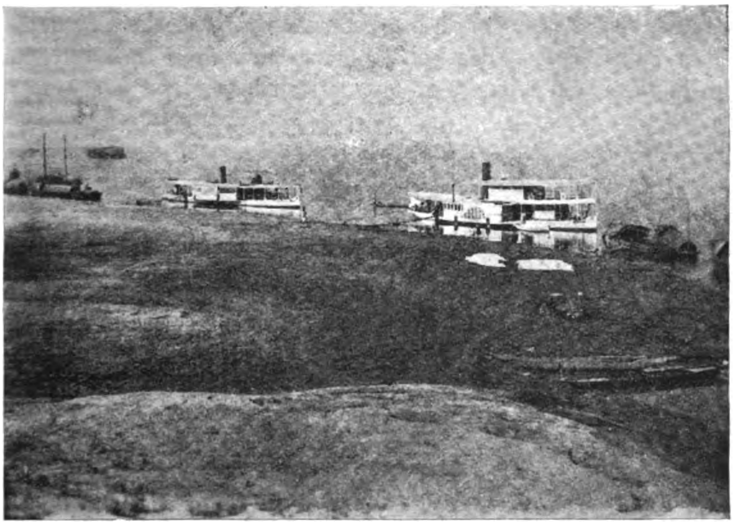

FIG. 6.—As the river goes down, pools for a time persist.

Perennial streamlets are invariably associated with a high spleen-index, which is proportional to the proximity of the habitation to the stream. In this the dangerous malaria-carriers *funestus* and *aconitus* are invariably found in abundance. The evidence for this conclusion was very strong in Sibsagar District,* where possible sources of malaria other than streamlets could very often be completely excluded. In Tezpur Sub-District we could not so definitely ascribe the high malaria rate of the Redbank gardens to the perennial streamlets as opposed to other elements in the situation : there was some doubt in our mind as to which streams run perennially.

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* On one or two estates, the spleen-index of lines near to such streams had very much smaller spleen-indices than one expected. Perhaps in these cases the spleen-index was not correctly recorded by the sub-assistant in charge.

Bhils,*—when under jungle or forest, discovered in them ***umbrosus***, and this must be the potential danger of jungly country. Probably on high ground where the rivers and streams are active, there is not much danger from ***umbrosus*** which, as stated elsewhere, is a still-water mosquito.

Bhils in some instances from which the influence of nearby perennial streams could not be dissociated seemed to augment the spleen-index above what one would expect would be caused by the streams alone.

Jans and bhils in the making are seen in Lakhimpur District in every stage of their life-history. In so far as they carry one or more grassy-edged perennial streams in their beds they are more or less malarious. Also other elements in their constitution must be considered, owing to the fact that their grade is usually low, and their outlet more or less silted up, the drainage of their catchment area accumulates in them and forms a swamp. The influence of the swamp probably depends on the rate with which the water is streaming through it and its permanency, yet it is never so easy to find *funestus* in the swamp as it is in the streams: on the other hand its extent is vastly greater. If the swamp be too deep, paddy cannot be planted in it, but in the slightly higher parts every available patch is cultivated. Evidence was obtained that a jan in which there was less swamp and more paddy, e.g., at Sookerating, was associated with less malaria than a jan which was a morass of wild vegetation, but it could not of course be said that this higher malaria rate was not due to more streamlets just as much as to more swamp. Another element in the situation is the seepage swamp at the foot of the banks which limit the jan, but only if these banks are high enough to provide a sufficient filter bed. Sir

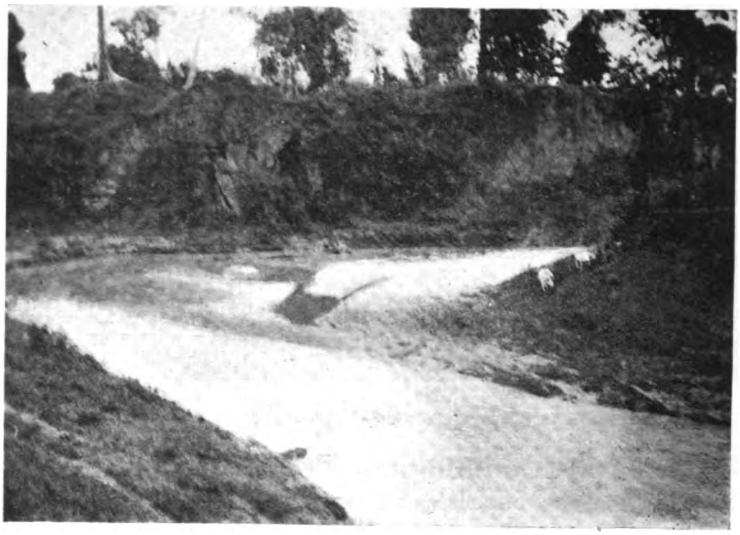

Fig. 7.—Rivers of the third magnitude.

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* Defined as having no or but slight surface outlet for their drainage system.

Malcolm Watson found numerous *maculatus* breeding in such swamps, and these might be expected to cause even considerable harm. However, the matter will be further discussed below. Sometimes the jan is practically dry all the year round and is really a *bhil* as defined above. Such a jan, e.g., the Maijan, acts merely as sump for a limited catchment area and in it paddy is extensively planted. It is very difficult to find any pathogenic anophelines in such places but they are associated with a lowish spleen-index of not more than 25 per cent.

Hulas. Shallow low-graded hulas and also sometimes highly-graded hulas may exist on estates which have a *low* spleen-index, while on many estates with a very high spleen-index there are many hulas in which may be found *funestus, aconitus* and either *maculatus* or *karwari.* There is some evidence, however, that these high spleen indices are caused rather by perennial hulas. On one very malarious estate the healthiest lines by far were situated near a hula and far from any permanent stream.

Swamps. The influence of swamps has been already hinted at above in so far as they are related to the features of the landscape which we have already discussed, but such permanent swamps as are found in places at the foot of the Naga Hills, in Nowgong District, do not seem to owe their origin to any river bed, but merely to considerable seepages from the hills behind (Fig. 8). The spleen-indices in their proximity are very high (round 100 per cent).

Spring-breeding anophelines. We have referred above to the occurrence of *maculatus* in springs at the base of the high banks which delimit the jans, and the problem now to be considered is to what extent is this species a serious menace to the estates. That a single specimen is undesirable must be admitted, but one must at the same time not neglect other and perhaps more important factors.

In the first place, the relative proportion of this species to the others over the whole of Assam was extremely low. In the preliminary survey, out of 2,774 specimens only 2 were certainly *maculatus,* while 15 were either *maculatus* or other species indistinguishable from it, while in the detailed survey there was only 1 possibility out of 2,049.

If, therefore, on any estate *maculatus* can be found in large numbers, the occurrence must be adjudged a purely local one probably due to peculiarly suitable conditions.

In this connection reference may be made to what was written on pages 11—12 under *maculatus.*

There is much evidence from all over Assam that *maculatus* is not a very important factor. Thus where lines are situated on a low bank near a big jan, e.g., Hapjan, and there is presumably a minimal amount of surface seepage, and therefore of *maculatus,* malaria may produce a spleen-index of 100 per cent; so it may be fairly argued that a (lower) spleen-index near to a big jan in lines on a high bank below which is much swamp and opportunities for *maculatus* to grow, (e.g., at Beesakopie), may be equally due entirely to the influence of the jan.

Then there are many estates planted on sites near to high banks at the foot of which there could have been no lack of seepage, but which were nevertheless very healthy.

And again estates otherwise similarly situated away from any obvious source of malaria except that one might be on a high bank and another on a low had approximately the same low spleen-index.

Or gardens situated on and at the margin of a great bank like the Tezpur Redbank had no higher spleen-index than those away from the edge of the bank.

The evidence is therefore considerable that seepage swamps at the foot of the banks and terraces on which tea is planted has no noticeable effect on the malaria-rate.

Jungle. The species *umbrosus* was found in the jungles of the Assam valleys in borrow-pits and in bhils. Moreover, evidence of its ravages was obtained. Its presence should be suspected on any low-lying ground on which pools of water filled with jungle débris may lie, such as the so-called pathar level especially where the soil is not of a porous nature. Where pools of water do not remain for any time the species is probably non-existent. As a matter of fact in Assam the malaria on nearly every estate might be ascribed to some other feature than adjacent jungle, for example, to a grassy perennial stream; very often lines near to jungle were no more unhealthy than if they were not near to jungle, so that in any particular case the harmfulness of the forest can probably only be demonstrated by finding an *umbrosus* production area in it and the adults in human habitations.

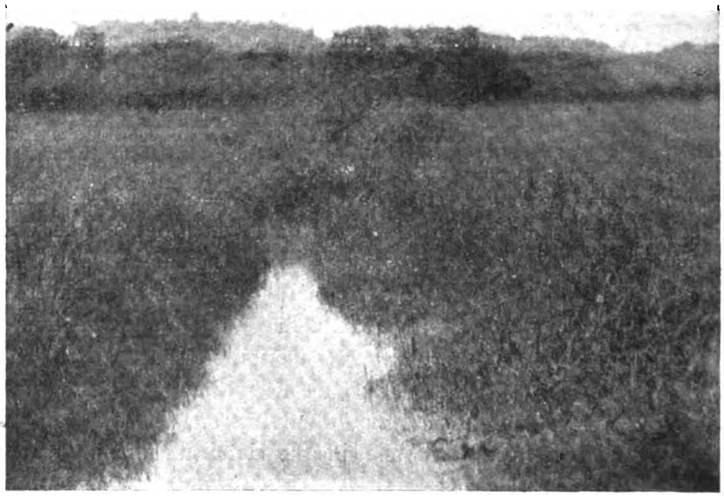

FIG. 8.—Swamps owe their origin to seepage from the hills behind.

That jungle is not always dangerous was proved by the fact that some comparatively healthy estates were surrounded by forest, and in it suitable breeding-places such as bhils or even hulas were markedly absent.

The fact was elucidated that on the pathar-level, where, as said above, if the land be under jungle, we would expect *umbrosus* to thrive, cutting down the jungle is an effective anti-malarial measure.

An interesting point, because one on which epidemiologists all over the world have found it difficult to obtain natural evidence, was that a belt of jungle interposed between a cooly line and a perennial stream seemed to protect the coolies very effectually.

Tanks. In so far as these are part of bhils their influence is the same, only less so, owing to the greater depth of their water content affording more scope for the operation of ' natural enemies '.

The Pathar. Estates situated on the pathar-level were comparatively healthy, one showed a spleen-index of only 6 per cent. The surrounding land was of course, always cultivated with paddy, the harmlessness of which with its myriads of *sinensis*, *vagus* and *fuliginosus* was thereby demonstrated. *

BB. THE SURMA VALLEY.

BBa. Cachar District.

Discussions on Groups.

BBa 1. Gardens 1 to 3 in the North-east corner. Pathemara, Goabari, and a Jainti village (Khasipunji).

In this group it is easy to understand the great amount of malaria in lines along the granite-bouldered hill-streams. It is due to *maculatus* but the reason for the fever on Pathemara Estate is not so evident. Perhaps the small river here breeds *maculatus* and *culicifacies* in the winter or spring or perhaps the swampy ground between the tilas breeds sufficient *funestus* to do all the harm.

BBa 2. Gardens 1 to 5 in the Bandhu group. Scottpore, Majargram, Doorganagar, Bandoo, and Thailoo Tea Estate.

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* In the original report after giving, as above, our conclusions regarding the malaria of the Assam Valley as a whole, we gave some data from Lakhimpur and Sibsagar Districts which pointed to certain district differences in the valley.

It was seen that Lakhimpur had a higher spleen-index than Sibsagar, 41·3 per cent to 37·2 per cent, taken in a very large number of children. The meteorological records showed that Lakhimpur had a considerably higher rainfall, and as the rainfall of the monsoon appears to be correlated with the annual malaria epidemic, one would expect that of the two Sibsagar would show less malaria.

On the other hand the two districts are markedly different in their physiography. Lakhimpur is in a greater state of riverine decay having reached a particularly dangerous stage. Whereas the process of decay has proceeded to a less extent in Sibsagar, and this may be the reason for the difference in the malaria endemicity.

The tables illustrating these differences are filed with the I. T. A., Calcutta, for reference if required.

In these gardens one common feature of the land was the high altitude of the bank on which the estates were planted, and the depth to which it had been sculptured by the hulas draining the bank. The seepage in these hulas from the overlying land must be considerable and might be expected to support a large number of the species *maculatus* and actually this species was found.

At the same time the banks have a number of shallow hulas coursing over them, e.g., at Thailoo, and this estate showed that such places are very dangerous even though presumably they do not carry perennial water. A large number of *funestus* were found in them. When they were dammed up nothing dangerous was found in the collected water.

What amount of malaria was caused by a large tract of rice fields such as that beside Thailoo through which coursed *funestus* streams was not ascertained.

BBa 3. Gardens 1 to 5 the Kasipur Group.

Cassipore, Digor-Kasipur (Felixstowe Tea Estate), Chappannahal, Arcuttipur, and Doyapur.

Of these estates the more malarious were those situated nearer perennial streams and conversely such an estate as Arcuttipur which had no perennial stream near it was comparatively healthy.

Arcuttipur showed that swampy ground or a small jheel on the pathar-level was not associated with a high malaria-index.

BBa 4. Gardens 1 to 4 on the 'Alipur spur.' Pallorband (Palairband), Alipur, Dooloogram (Dalugram), and Phanree.

These estates, all apparently with a very similar rate of malaria, have their lines near to some perennially running stream. It should be noted that whether the lines are on high land among the tilas as at Dalugram, or on low banks as at Alipur, or on the flat as at Pallorbund, the resultant malaria is very much the same.

BBa 5. Gardens 1 to 7, the Tarapur Group. Tarapur, Lallang, Labac-Bill (Bheel Tea Estate), Dewan Tea Estate, Burtool, and Lydiacherra.

The very high malaria-rate of Tarapur must be ascribed to the very close proximity of one stream, as well as to others carrying perennial water. The tea-bank is high and possibly there may be *maculatus* 'breeding' grounds at its foot. Moreover, there is permanent swamp in the vicinity.

Labac, Burtool, Dewan, and Lydiacherra have a spleen-index of between 50 to 60 and all have the surface of the tea bank with grassy hulas in which *funestus* are found. Moreover, Labac and Dewan are in relation to a wide tract of khet land in which run permanent streams.

Bheel is very interesting as an example of a very healthy estate planted on a tila surrounded by land at the pathar-level. Some of this has been very well drained and set out with tea, while the rest of it is used for dhan-khets. Here, then, khet lands are not particularly dangerous.

BBa 6. Garden 1. Hillara Tea Estate.

Granting that its reported spleen-index is a true representation of facts, this garden is an interesting illustration of the comparative innocuousness of permanent swamp and bhils in the bhil country.

BBa 7. Gardens 1 to 4 in the Lallacherra Group. Lallacherra, Durgapore, Vernerpur, and Roopacherra.

Lallacherra, Vernerpur, and Roopacherra are interesting as showing the influence of small streams and jhils on the malaria-rate, in the bhil country; the rate is comparatively low.

Durgapore is away from any permanent stream but surrounded by low drains and nullahs which dry up after the rains and on this estate the spleen-rate appears to be still lower.

BBa 8. Gardens 1 to 5, the Aenekhal Group. Aenekhal Tea Estate, Luckynugger, Monacherra, Old Singalla, and Ramchandi.

The 'key' garden in this group is Monacherra. It lies on a mass of tilas rising up like islands from the sea of 'bhil country' around it. It is a comparatively healthy garden and has a low spleen-index. It shows that the streams and the bhils of the bhil country are not productive of a high spleen-index.

The higher malaria-rate on the other gardens planted on the tilas of the main hill range cannot therefore be ascribed altogether to the Dhaleswari which runs near them but is probably due mainly to the perennial streams which are tributary to that river. In some of these *funestus* was captured. This hypothesis is supported by the fact that on Old Singalla the nearest perennial water is half mile away and the spleen-index is comparatively low.

BBa 9. Garden 1 in the hills south of Silchar. Katal.

This estate only illustrates the rather low malaria-rate associated with land at the edge of the bhil country near to a small river, a mass of bhils, and to perennial swamp, but where there are no perennial streamlets.

BBa 10. Gardens 1 to 3 in the Jatinga Valley Group. Jatinga Valley, Sahazpur (Shabashpur), and Bicrampore Tea Estate.

Jatinga Valley and Shabashpur are both very malarious estates, as one would expect from their situation above the mountain stream. *maculatus* and perhaps *culicifacies* are doubtless the cause of the high spleen-index here.

Bicrampore, on the other hand, seems to be of the bhil-country type.

BBa 11. Gardens 1 to 5 in the Haticherra-Dudpatli Group. Haticherra, Tamacherra, Chaltakandy, Kannykoory, and Dudpatli.

Haticherra and Tamacherra are both considerably malarious and this is probably due to streams of permanent water coming down from the heart of the tila country, as well perhaps to springs of water seeping out of the hill-sides. On Haticherra there is a big bhil apparently due to the blocking up of the valley of one of the streams; the influence of this on the malariology of the place was not ascertained.

The other three gardens, Chaltakandy, Kannykoory, and Dudpatli are less malarious, although they all lie under the influence of permanent streams. This point will be reverted to later.

The swampier the surrounding land becomes and the closer to bhil, the less malarious lines be.

Further, the least malarious of the group are those of Dudpatli along the high banks of the Barak.

BBa 12. Garden 1 in the Katakhal River Area. Barni Braes.

There are apparently no streams of permanent water in the neighbourhood of the lines but a good deal of permanent swamp lies about in addition to the bhils. It is not clear how much of this swamp is due to seepage from the hills behind, but probably whatever excess of malaria there is on the estate over the amount caused by the bhils will be due to this permanent swamp.

BBa 13. Garden 1 in the south-east of the Barak River. Roopabally.

This estate lies in the heart of the bhil country and apparently has a comparatively high spleen-index. If this be correct, one must assume that it is due to the very big bhil beside which the estate is planted.

BBa 14. Gardens 1 to 5 in the Massampur Group. Massampur, Khoreel, Kuna Tillah, Kallinugger, and Moolydar.

In this group, Massampur shows the influence of the Barak and small jheels combined. The other lines lie near to perennial streams, and the spleen-indices vary from 35 to 60 per cent.

BBa 15. Gardens 1 to 4 on the Badarpur Spur. Badarpur, Margonpur, Chandypore, and Serishpur.

Badarpur Tea Estate shows that the Barak is not very harmful, nor the bhils which lie here and there near the lines, nor the hill-side streamlets which dry up after the rains.

The other estate lines are all near perennial streams and jheels. Serishpur has permanent swamp too (compare Barni Braes).

CONCLUSIONS.

Regarding Malaria in Cachar District.

It will have been seen above under the discussion on each group that the malaria of the gardens in Cachar can often be definitely ascribed to certain features of the terrain.

Thus gardens planted on the lower slopes of the Khasi-Jaintia Hills through which rivers and streams with all the characters of mountain torrents force their way, have a very high spleen-index, around 100 per cent. Such a garden is Goabari above Pathemara. The explanation of this high rate is doubtless that such sites are very favourable to the evil carrier-mosquito *maculatus;* and perhaps also *culicifacies*, though the latter was not actually found.

Small and moderate-sized active rivers on 'the plains' like the Dhaleshwari or Katakhal have been proved not to be associated with a high spleen-index. What comparatively low spleen-index is found in their vicinity may perhaps be due, and probably is, to other elements in the local scenery.

The same remark applies to the great river, the Barak. The low spleen-indices found in its vicinity are quite probably due to other factors; a matter to which we will revert below.

Turning back now to the tributary streamlets of these rivers on the 'plains' it is proved on one estate (Monacherra) that when the streamlets are *non-perennial*, they are not associated with a high spleen-index, but to what approximate extent the moderate index of about 25 per cent found in their proximity may be caused by them or by some other factor in the locality cannot be stated.

It is a different matter when the streams carry perennial water in the grassy edges of which *funestus* can always be found. In group after group their danger was demonstrated, the nearer an estate to a perennial stream the higher its spleen-index, which was found ranging up to 100 per cent, probably proportionate to the distance of the habitation from the stream. There is some evidence that the grade of permanent stream is an important factor. This must be so when one considers the non-malariousness of a meandering river like the Barak and the high malariousness of a grassy-edged stream as at Tarapur. An example is seen in the spur of hills at the point of which is Dudpatli. The nearer to the point of the spur where the *tilas* are very often discrete and rising out of the plain like islands and the streams have reached the pathar-level the less malarious are the estates.

In deep hulas on the *tila* country, e.g., Scottpore, Bandhu, etc., another factor is probably in operation. The species *maculatus* is found in their impermanent springs and is associated with a very high spleen-index, round 100 per cent, in spite of the water not running perennially. In one estate, Thailu some lines placed near a *non-perennial* stream of spring water in a very shallow rocky hula (*funestus* found in numbers) had a spleen-index of 100 per cent, a much higher rate than is as a rule found near *non-perennial* streams. Perhaps the explanation of the discrepancy lies in the fact that the lines here are so very close to the hula or alternatively that *maculatus* (which, however, was not found) may be an important factor in this rocky ground, a situation to be compared with places like Scottpore and Bandhu.*

It was noticeable in many groups that the situation of lines on different heights of banks or tilas did not seem to influence the spleen-index at all. This suggests that *maculatus*, which is essentially a seepage breeding anopheline, is not a very important, if any, factor in the mass of estates, for a high tila or a long range of *tilas* would naturally have more spring water at its foot than a low *tila*, with corresponding opportunity of breeding *maculatus*.

This matter leads to the question of the culpability of permanent swamps which may be found near, or on, only a few estates.

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* It has since been ascertained that a permanent stream runs not far off.

There is definite evidence in several places that permanent swamps mixed up with bhils in the 'bhil country' are not associated with a high malaria spleen-index, the average probably being about 25 per cent, and of this what proportion is due to the swamp and what to the bhil cannot be said. On some gardens a permanent swamp closely related to perennial streams was associated with a very high spleen-index, but in this case, of course, the streams could be held largely responsible. On the other hand two estates, Barni Braes and Serishpur, both with a spleen-index round 50 per cent had hill-foot permanent swamps near their lines, in the latter estate as apparently the sole source of infection; in the former the swamp mixed up with a small bhil. The explanation for this higher spleen-index must be that the swamp was of spring water from the hills behind, the swamp water running away in grassy streamlets. In general it would be safe to conclude that swamps behave in an analogous way to streamlets on the one hand and bhils on the other, as will be described below.

In only two instances in all of the many estates of Cachar which were visited were bhils associated with a high malaria-index. In one case (Roopabally) a very high spleen-index was reported by the assistant in charge and the only possible source of this malaria was a very great bhil of permanent water. In the other instance, that mentioned above, at Barni Braes, a permanent swamp and a small jhil together were apparently responsible for a spleen-index of about 50 per cent.

Although the Roopabally figures require confirmation, there is no inherent improbability of a great bhil producing a 100 per cent spleen-index, if a small one will produce a small index. The index depends on mosquito production and this on opportunities of 'breeding,' one of which is space. A few *aconitus* and *funestus* can always be found in any bhil. But size for size, the state of a bhil should determine its malariogenicity. A bhil is a river bed not functioning as such or only slightly. Every stage can be seen from a sheet of water which cannot be strictly called either bhil or river, through the condition of a grassy marsh to one of complete dryness and suitable for paddy cultivation. It is obvious that the more stream-like the condition, the more dangerous the bhil.

Yet except in the two instances above mentioned the spleen-index associated with the bhil country was low, on an average about 20 per cent.

Dams and Drains. A tea-bank hula, normally a habitat of *funestus*, dammed up by a high bund for conservation of water, did not discover any pathogenic anophelines.

In several instances in which there was a low spleen-index the tea land was drained, so that the drains could be considered nearly harmless. A typical instance of this was on the Bhil garden of Labac. The lines here are perched up on a tila arising like an island from the surrounding plains level. Part of this is drained and planted with tea while part is undrained and devoted to paddy cultivation.

Paddy Cultivation.—The last instance shows what a little ill-health may be caused by paddy cultivation on the pathar-level. Here, however, ran no permanent stream. The streams which irrigate rice fields are certainly dangerous and probably the rice fields themselves, i.e., the land *minus* the streams, also is to a certain extent, if the water lying on them is spring water, spilling away. *aconitus* and *funestus* can often be found in them, though it is the irrigation channels which are most dangerous.

BBb. SYLHET DISTRICT.

Discussions on Groups.

BBb 1. Gardens, 1 to 5 in North Sylhet. Doldoli, Lakhatoorah, Malinicherra, Kewacherra, and Khadim-naggar.

The estates in this group have a relatively high spleen-index. Swampy ground and bhil areas are noticeably absent and the malariousness must be ascribed to the streams.

BBb 2. Bhil country near Banyachong.

The comparatively low spleen-index associated with this typical part of the bhil country must be noted. To what elements in the terrain that index was due cannot here be stated.

BBb 3. Gardens 1 to 7 in the Rajkhandi Hills of South Sylhet. Alinagar, Suncherra, Bhagicherra, Chatlapore, Shamshernagar, Daocherra, and Kanihatti.

In considering these estates, one sees the influence of bhils and kals represented in Alinagar with a spleen-index in all its lines of about 20 per cent.

Hula streams and khets and bhils play their part in Suncherra with its higher spleen-index of about 50. One might conclude that Alinagar has a generally low spleen-index as it is practically on the pathar-level, but so are Daocherra and Kanihatti with higher spleen-indices of about 50, examples of what a perennial stream on the pathar-level can do.

Bhagicherra is very interesting as it shows what a very high endemic index is associated entirely with hulas and streams among the hills.

BBb 4. Gardens 1 to 5 in the Doloi Valley. Podocherra, Madabpore, The bustees on the khet land, Patrakola, and Champarai-chara.

One particularly interesting thing stands out from the consideration of what was found in these estates, to wit the absolute harmlessness of khet land in certain circumstances, and also of a wide active stream such as the Doloi. In Patrakola New lines one may take the spleen-index of about 30 as due to the influence of either the small bhil which is close at hand or to the comparative proximity of other dangerous features in the terrain. The same remark applies to the Nagbasi lines on Champarai-chara.

The old lines on Patrakola are also interesting as demonstrating the danger of a small stream (the Gulguli) running near the lines.*

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* In a subsequent survey here it has been ascertained that there is another cause.

The other gardens do not afford any evidence of the relative harm of the bhils and streamlets.

BBb 5. Gardens 1 to 5 in the Balasera Valley (near the railway). Baraoora, Jagcherra, Ghellacherra, Bhurburipur, and Kylcherra.

These estates show very variable spleen-indices probably dependent on the relative position of the lines to the streams and bhils.

BBb 6. Gardens 1 to 4 on the Balasera Hills (south of the railway). Kakicherra, Phulcherra, Kalighat, and Luckicherra.

Luckicherra lying on higher ground seems to be more malarious than Kalighat or Phulcherra which lie on very low banks. Luckicherra is probably influenced by the jhil in which many *funestus* were found or by the higher grade of the land while the other estates seem to have been infected mainly from streamlets and grassy kals in all of which *funestus*, *aconitus*, *karwari*, etc., could be obtained.

BBb 7. Gardens 1 to 9 in the Balasera Valley (central group). Rajghat, Udnacherra, Burmacherra, Kajuricherra, Sisalbari, Fuskuricherra, Putiacherra, Schinderkhan, and Tipperacherra.

One thing that all of these estates have in common is their uniformly very high spleen-index and reputation for severe malaria.

The general lie of the land is exceptional in the case of Tipperacherra which is mostly planted on high tilas, while in all the others the banks of tea present only a flat or gently undulating surface and constitute small plateaux with good drainage. This shows that the height of the tea land in itself is not a determining factor in the incidence of malaria.

In all these estates the banks of tea land or the tilas have intervening kals which may be either grassy swamps or planted with paddy or occupied by swift-flowing streams. In these situations numbers of *funestus* (*minimus*) could always be found. There are also in this area, including Tipperacherra at its debouchment on to the plateau, a number of bhils and permanently swampy tracts occupying the sites of old river beds and in these it was quite common to find *aconitus* and *funestus*. These species were also found on one occasion in a tank with grassy edge.

A very interesting discovery was the great number of *funestus* captured in a stream running through a wide tract of khet land which was at pathar-level, which here then must be viewed with suspicion.

BBb 8. Gardens 1 to 4 on the western side of the Balasera Valley. Satgaon, Gandicherra, Amrailcherra, and Hooglicherra.

The spleen-index of Satgaon is comparatively low (if the figure given can be accepted as reliable). It is understood the surroundings here are very well drained and water never lies for long, which may account for its low malarial condition.

The other gardens all have a very high spleen-index. Gandicherra lies on very low banks drained by a few streams, one of which runs past the lines. Amrailcherra lies on higher banks, while Hooglicherra is planted on still higher tilas. The height of the tea land therefore does not here seem to determine the spleen-rate. In each case there are a number of bhils and swamps to which as to the streams must be attributed a certain proportion of the malaria endemicity.

BBb 9. Gardens 1 to 4 in the Sutang or Khowai Valley. Chundicherra, Chandpore, Begumkhan, and Luskerpore.

An interesting point arising in this group is the very high malaria-rate in two estates which are planted on very low banks with very shallow intervening kals. The fall, however, in these is good and the beds silty. Many of the kals were planted with rice; *aconitus* and *funestus* were found universally in these places and must be accounted responsible for the high malaria-rate. These kals and the Chundicherra stream into which they drain are continued over the pathar-level and empty into the Sutang and carry these dangerous species there too. Another interesting point about these places is that no jhil system appears to have been developed hereabouts, so that the kals and streamlets alone are probably responsible. It must be noted that the Chundicherra in Chandpore is a perennial stream.

Luskerpore, on the other hand, is planted on high tilas on which are the lines, except for one set beside a perennial stream and that is very highly infected. All the lines show a very high endemic index and this must be ascribed to the swampy or paddy-grown kals where malaria-carriers are prolific.

BBb 10. Gardens 1 to 4 on the western side of the Shaistaganj Hills. Surma, Teliapara, Satchurie, and Noyapara.

This group of estates lies along the foot-hills where they debouch into slightly undulating land. There was no apparent correlation between the height of the tea-banks or tilas and the spleen-indices:. Teliapara, however, with a uniform 100 per cent spleen-index lay on high tilas.

Comparative lack of perennial water about all these estates was a characteristic point noted.

The bhil system was rather in evidence and the bhils nearly always contained pathogenic anophelines. For the rest there was no evidence as to the relative importance of the sandy bedded active streams and streamlets and the bhils.

CONCLUSIONS.

Regarding Malaria in Sylhet District.

A review of the notes made above concerning the salient characteristics of the terrain conducing to malaria in Sylhet leads one to conclude that probably everywhere in this district proximity to perennial running grassy-edged streams are a most important factor, the reason without doubt being that here is the site of election in the larval state of the notorious malaria-carriers *funestus* (*minimus*) and *aconitus*. Another mosquito which may possibly contribute to the malarial incidence here is *culicifacies*, though this species was not certainly identified in our survey. It might conceivably be found in pools on sandy beds left at the end of the rains by the receding streams, indeed it was very noticeable how in the South Sylhet estates with such streams near their lines the spleen-index was very high, sometimes 100 per cent.

But in Sylhet such conditions are not so commonly met with as are grassy-edged streams, and in the former, too, *funestus* is common enough.

These streams are, of course, often used for the irrigation of *dhan-khet*, but we cannot recall ever having found any one of the notorious malaria-carriers actually in the khets (or fields as distinct from the irrigation system).

The spleen-indices of lines where grassy perennial streams have been incriminated by the exclusion or absence of any other possible factor have varied from about 50 per cent to 90 per cent. This variation is no doubt due to varying distance and local differences in the edge of the stream. Presumably a minimal amount of malaria might be due to such a source acting from a maximal distance.

The '*dhan-khet*,' or pathar-level, is so commonly said not to be associated with severe malaria that it is interesting to find that where a grassy perennial stream waters the land, it may be the source of a considerable malaria-index to people living in its proximity, in fact just as high an index as there may be in those living in the more undulating parts.

The fact of the matter is that the height of the land on which the tea is planted and on which the coolies live does not matter a tinker's curse. This is proved time and again in South Sylhet. Two estates planted on low banks like Phulcherra and Chandpore may have spleen-indices as wide apart as 20 per cent and 80 per cent. At the same time the grassy streamlets watering the hills seem to be invariably associated with a high malaria-rate (e.g., Bhagicherra). This perhaps gives a clue to the variability in health on the 'plains': where the fall is slight, the flow sluggish, and the stream swinging (meandering) in its valley bed, conditions associated with 'dying,' (seen in the grassy kals at Phulcherra and Kalighat), the conditions are not so favourable for the pathogenic mosquitoes and so the malaria-rate is less. It must be nevertheless pointed out that *funestus* and *aconitus* are mosquitoes of catholic tastes (*see* Appendix) and are found in the circumstances just described, and moreover *karwari*, a reputed carrier, is another species commonly found in the same places.

Before now proceeding to consider the influence of bhils on the malaria-rate, a subject naturally led up to from the above mention of that of decaying streams, it must be pointed out that big active rivers and streams are for all practical purposes innocuous, for example, the Doloi River in South Sylhet. Its present bed is almost fully occupied by the stream, its side banks and bed are almost devoid of vegetation (except for a reedy grass), owing doubtless to the silt deposit and floods of the stream, and the consequence is that for all practical purposes there are no possible breeding-places in such a site.* A smaller stream like that at Kalighat is associated with a slight malaria-rate, though it was not proved that this was due in any way to such a stream.

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* Watson found *maculatus* breeding in a pool in the bed of the Brahmaputra at Dibrugarh but notes that such a river is not associated with malaria to any extent.

With regard to rivers, streams and streamlets in general, it would probably be safe to conclude that in their most active state they are harmless; when in the condition which denotes adolescence and senescence they are very dangerous, and with decay they gradually lose at least most of their harmfulness.

The evidence afforded by Sylhet is insufficient to enable us to come to any very definite conclusion as to the danger of bhils. These, of course, are the end product of the decay of streams, and represent their old valley-beds. Usually they only contain water during the rains, that dry up during the hot months, though they are like tanks in respect of the permanency of their water—in fact tanks are often constructed in bhils. They often hold *funestus* and *aconitus* beside the usual 'non-carrying' anophelines.

One thing can however be definitely stated with regard to bhils, and that is in no instance has a higher spleen-index than about 30 per cent been found associated with them and as stated above it is impossible to say in any case what proportion of this was due to the bhil. The difficulty about assigning any degree of harmfulness to bhils is due to the fact that they nearly invariably lie close to the streams which have sidetracked them, and consequently any ill-health in the neighbourhood may be due to both in varying proportions.

MALARIA IN THE SURMA VALLEY.

If we compare our conclusions from Cachar and Sylhet Districts regarding the correlation of malaria with certain elements of the physiography, we are led to consider that there is much true of each district which is true also for the whole valley.

Let us first review the antithetic cases of active streams and bhils.

Active streams, whatever their size, are confined to a channel which they have made for themselves and which pursue a course the sinuosity of which is dependent on the fall of the stream. The amount of water (Fig. 9) which they contain is a measure of the part of the rainfall which has not been absorbed by the earth in the catchment area of the stream, plus the seepage or spring water which crops out in the same area, and the total may vary from nil to such a quantity that the river channel is filled up and overflowing. When there is a minimal quantity, inequalities in the bed of the stream may lead to pools of water being left behind by the recession of the stream.

In the Surma Valley a great and active river like the Barak was found not to have a high spleen-index associated with living near to it. The spleen-index was round 25 per cent and this was probably due to other elements in the local scenery which could not be dissociated from the problem. The rise and fall of such a great silt-laden river prevents the growth of any vegetation along its banks and it is hopeless to look for mosquitoes along its glistening mud-smoothed course. Probably it is responsible for no malaria at all.

Moderate-sized active rivers like the Dhaleswari or Katakhal might have the same remarks made of them. They are but the Barak writ smaller.

These conclusions regarding rivers must, however, be restricted to those on the pathar-level, that is to those on the lowest plains which in Cachar comprise "the bhil". Here the gradient of the land is low, the rivers oscillate perhaps in semicircles or even more acutely, the land is liable to flooding in the rains or its drains may be subject to back flow from the rivers, the subsoil water is high, and towards the end of the rains paddy covers the land. Malaria mosquito-carriers on this sort of land are difficult to find, *vagus* is the commonest mosquito found in any pool of standing water, while *barbirostris*, *sinensis* and *fuliginosus* occur in swamp in the flooded paddy fields.

Some, but slight, evidence was collected during the survey to justify the conclusion that the smallest streams meandering over the pathar and, due to their serving a minimal drainage area, suffering greater periodic variations in the amount of water they carry, usually drying up after the rains and therefore being edged by grass and weeds, are like their larger sisters in these regions, not associated with a high spleen-index, e.g., the *non-perennial* streamlets near Monocherra which are associated with a spleen-index of about 25 per cent (this, of course, also possibly due to some other element in the scenery).

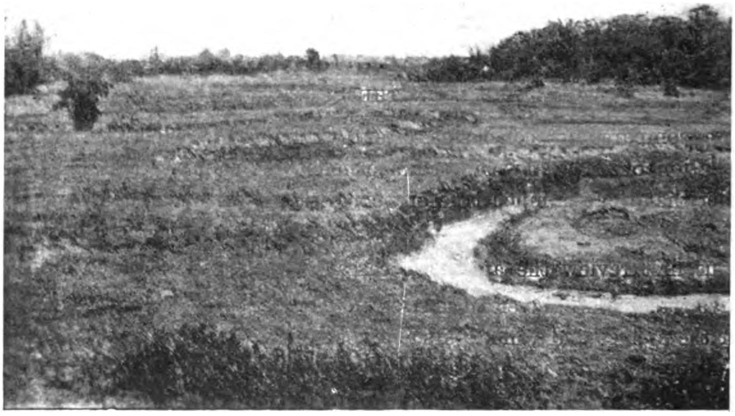

FIG. 9.—A course the sinuosity of which is dependent on the fall of the stream.

And with them we may lump the artificial drainage channels cut for the purposes of man, e.g., at Labac Bhil Tea Garden (Fig. 10). In such sites, mosquitoes are much more readily found than in big rivers because of the grassy shelter, but harmless the species are, usually.

There is, however, certain testimony that when grassy perennial streams run over the pathar, people living near to them may suffer considerably from malaria (spleen-index round 50 per cent) and in such streams *funestus* and *aconitus* can easily be found. In such cases we feel doubtful whether it is the perenniality of the flow which is the dangerous element in the matter or the fact (which was observed in the cases in mind) that there was a higher gradient, clear fast-flowing water and a silty bed in the streams, which conditions seem to be more to the liking of the species *funestus*. We are inclined to the latter hypothesis on the strength of the following evidence. Perennial streams, arising from the range of hills and tilas to the north of the Barak River at the toe of which is Dudpatli Tea Estate, are associated with an ever-decreasing spleen-index in the estate lines situated on the spur, the nearer the streams, with an always lessening gradient, come to the pathar-level.

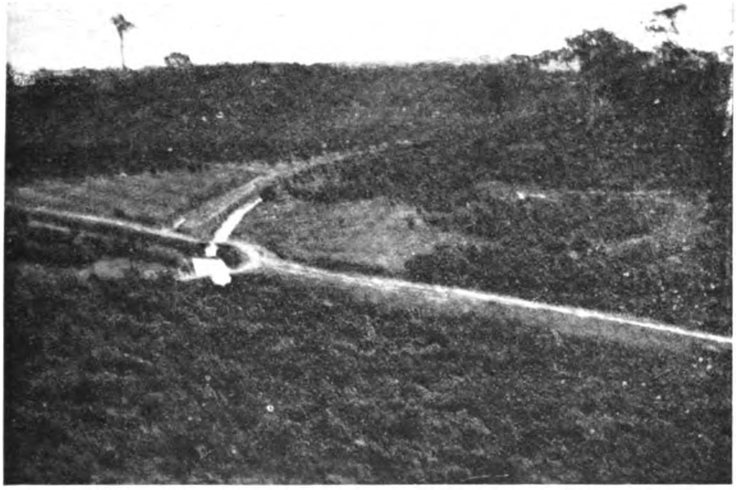

Fig. 10.—Artificial drainage channels at Labac Bhil Tea Garden.

If we now ascend to the higher levels and terraces of the Surma Valley, we again find, in such big active tributaries of the Barak as the Dholoi, that in people living near to them malaria is non-endemic. The Dholoi is on a steeper grade than those already discussed and its bed is of a silty nature.

A smaller active stream, e.g., the Kalighat, also running on a silty bed, is associated with a slight malaria-rate (Fig. 11), though in this case it could not be proved that this was due to the stream. Such a medium-sized stream would seem to us to be potentially dangerous when it is drying up in the autumn and leaving pools in its bed which might grow *culicifacies* and *maculatus* (*cf.* the Lumding stream, *see* p. 11).

It is a different matter when we come to analyse the effect of the small grassy-edged perennial streams in which *funestus* and *aconitus* can always be found in great numbers. In group after group of estates, both in Cachar and Sylhet, which we have analysed, their danger has been demonstrated; the nearer lines are to such perennial streams the higher the spleen-index which ranges from round 50 per cent to 100 per cent. Tarapur Tea Estate in Cachar provides an excellent example of such conditions.

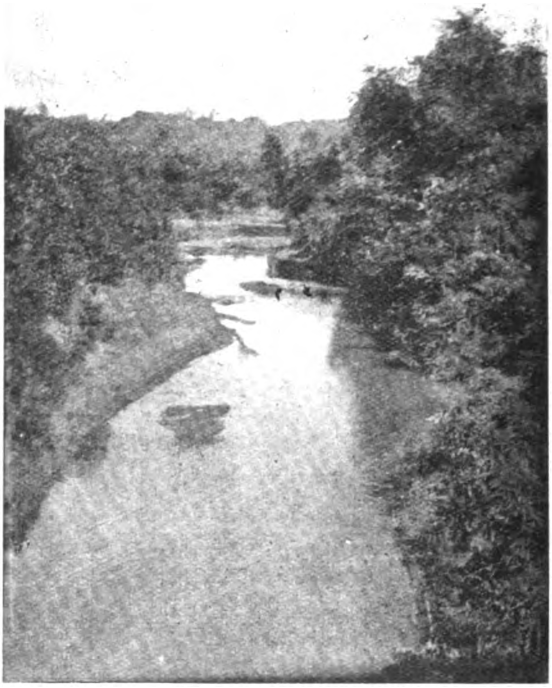

FIG. 11.—A small active stream is associated with a slight malaria-rate.

If we go still higher in the sides of the valley we have mountain and hill streams dissecting their courses through the rocks on the way to the bed of the valley. The estates planted on the foot-hills of the Khasia-Jaintia range, for instance, have a maximal spleen-index most probably entirely due to the species *maculatus* which breeds in pools of clear running water in mountain streams (and springs).

Turning now to the antithetic conditions of the bhil, which is, of course, the end product of the decay of streams (short of accumulating material and becoming levelled up to the plane of the surrounding land), the bhil representing the old valley bed, it is found that they too often hold *funestus* and *aconitus* beside the usual non-carrying anophelines. Tanks are often constructed in such sites and what is true of a bhil is true of a tank (though less so, for the comparatively clean cut edges of the latter and the usually greater depth of its more permanent water favours the operations of animal enemies of mosquito larvæ).

Bhils in Sylhet were never found associated with a higher spleen-index than about 30 per cent and a certain proportion of this, if not all, might perhaps have been ascribed to other proximate conditions, e.g., the perennial streams which have side-tracked them. No differential effects were noticed in bhils varying in the permanency of their water content. In Cachar, in one instance, a very high spleen-index was reported to be associated with a very large bhil of permanent water. There was no inherent improbability in the report, though it was in our experience such exceptional an event that one would like some confirmation before finally accepting it. In another instance, there was a rather high spleen-index of about 50 per cent associated with a permanent hill-foot swamp and a small jhil. Probably one may fairly conclude that in this case the hill-foot swamp was largely responsible and in the Surma Valley in general that the average bhil near or on an estate does not effect more than a low spleen-index, and this even where its waters are permanent.

Swamps may be conveniently considered here, or such of them as only represent the sumpage of a small catchment area into a bhil bed. It is quite obvious that between the condition of a wide expanse of deepish water in a bhil which may overflow during the rains, and the same site filled up to the level of the surrounding country by alluvium, there is a state which is conveniently called a swamp. As this connotes a great deal of light herbage, reeds, and grasses, all of which are conducive to the livelihood of anophelines, one might imagine that such a condition would be one of great danger, but there is definite evidence in several places that it is nowhere associated with a high spleen-index (one round 25 per cent).

Now between the two extreme states which we have selected for our notice of the active river or streamlet and the dead river-bed or bhil, there is a whole range of intermediate conditions all arising from the fact that active rivers tend to carve out for themselves wide valleys in which the stream oscillates from side to side, some of the beds of which become separated off by alluvial deposits and then constitute bhils.

We will consider a few intermediate forms; hulas are valleys varying in depth from the slightest depression on the general surface to one of profundity, at the bottom of which runs an active stream. This carries off the storm water of the immediate neighbourhood and as much spring water as comes to it from the head of land above, the resultant determining whether the stream be perennial or not. Usually hulas do not carry perennial water, the springs drying up gradually after the rains stop. In so far as hulas carry perennial water in their active streamlets they are harmful just as are the perennial grassy streamlets mentioned above. If not perennial they nevertheless carry a great number of *funestus* and *aconitus* in their grassy edges during the rains, the point remaining how much harm do these effect, a point on which no evidence was collected in the Surma Valley.

But a hula is a complex of conditions of which the active streamlet is only one. Another important characteristic is the spring water which sometimes oozes out of the sides of the hula and trickles down to its bed and so into the stream. This spring water, of course, depends on the amount of water which has soaked into the column of earth which lies above the hula. If there be any seepage, it is very likely to provide a home for *maculatus* and must be viewed with great suspicion. In the very deep hulas of some of the hilly estates in Cachar, e.g., at Scottpore and Bandhu, *maculatus* is found and the spleen-indices of such estates is very high. Although seepage is most likely to be found in each deep hula with its extensive earthen filter bed above them, yet springs may be seen also in shallow hulas, for instance, where there is impervious rock not far beneath the surface. All springs must be viewed with some anxiety because of the likelihood of their supporting *maculatus*. One estate visited in Cachar, viz., Thailu, had a set of lines near to one of these shallow rocky hulas with many springs in it and the spleen-index was near 100 per cent.* No *maculatus* was actually found, but it might be strongly suspected to have its habitat there and to be responsible for much of the high spleen-index.

In general, we are inclined to believe that where *non-perennial* water is all that occurs in a hula and there is a very high spleen-index of nearly 100 per cent, then *maculatus* is to be found and to be largely responsible.

Another point in connection with hulas must be mentioned, that they are used extensively for paddy cultivation in small plots. The water supply is efficient, for not only is the hula the bottom of the catchment area for the rainfall, but there is the streamlet to use for irrigation by artificial irrigation channels and finally the earth on its bed is often soaked with spring water. Except that the irrigating channels are for all practical purposes active streamlets breeding *funestus*, though probably never perennial, the paddy culivation has probably no ulterior effect.

When a hula has been degraded to such an extent that the bed has only a very slight fall, the fact that it acts as a sump for the local rainfall which cannot easily flow away often leads to it becoming a deep swamp choked with grasses and weeds and unsuitable for paddy cultivation, e.g., at Phulcherra in Sylhet. Such hulas where the grade of the stream is low and the flow sluggish and wherein is much stagnant foul swamp (although they support *funestus*, *aconitus* and *karwari*) are not associated with a high spleen-index, one of about 20 per cent. They are river beds *in senescente* and it will be remarked that their effect is comparable to the young active low-graded meandering streams on the pathar. Grade seems again to be the important point.

Swamps. We have already referred to swamps above, first as a synonym of certain bhils, secondly as seepage outcrops in hulas, and finally as sumpage into hulas so lowly graded that it is retained and become foul and stagnant. Really, the first and third are of the same essential nature and their effects similar. In cases where there is permanent swamp closely related to perennial streams and an associated high spleen-index, this, of course, is ascribable to the perennial stream.

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* This particular hula was reported not to carry permanent water in it. It was a fairly big one and might very well have done so at any rate a little below the point examined: in which case the proximity of the lines so close to a perennial stream in which indeed great numbers of *funestus* were found is explicable *per se*.

It only remains to mention that in our survey exceptional instances of a comparatively high spleen-index (about 35 per cent) were recorded which were apparently due to permanent swamps at the foot of a range of hills abutting on the pathar. The swamp water was apparently of spring water from the hills behind and this was running away, so that the swamp might be considered quite analogous to grassy edged perennial streamlets, and the effect similar.

Malaria on Pathar or Tila. In the Surma Valley it was a very striking fact that with certain exceptions already adverted to, the situation of lines on different heights of banks or tilas above the pathar, or on the pathar did not seem to influence the spleen-index at all. Two estates (Phulcherra and Chandpore) on equally low banks adjacent to the pathar had spleen-indices as wide apart as 20 per cent and 80 per cent. There could be no question on such estates of the intervention of *maculatus* in seepage swamps at the foot of these low banks for there were no such swamps, so that it can fairly be agreed that a high spleen-index or a low one may equally well be seen on estates planted on high banks and may likewise be altogether due to other factors than the *maculatus* one.

Paddy Cultivation. This *bête noire* of sanitarians is involved in all that has been said above regarding rivers, drains, bhils, hulas, swamps, and so on, but it is as well to note in this place that paddy cultivation is only dangerous in so far as the land on which it is found would be dangerous if it were not cultivated.* Thus paddy grown in a bhil would be associated with the same sort of spleen-index as the bhil uncultivated.

In the original report, the above conclusions were succeeded by our Appendix (No. IV B) illustrating differences between Cachar and Sylhet Districts. This Appendix will be filed with the I. T. A., Calcutta, for reference, if required.

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* Probably the formation of irrigation channels where were none before would make matters worse.

PART V.

GENERAL CONCLUSIONS REGARDING MALARIA IN ASSAM TEA GARDENS.*

If we were to submit in detail our conclusions † regarding the malaria in the Assam Tea Gardens, the procedure would involve much repetition of what has been written above relative to the two great valleys, and such repetition would appear to be scarcely justified, for no particular distinction between them can be traced in any important character.

The reader is therefore invited to refer to those chapters on Malaria in the Assam Valley and in the Surma Valley on pages 78 and 93 respectively.

The conclusions drawn there are supported by those obtained from the analysis in Part III of the seasonal incidence of malaria and mosquitoes, and this section may also be referred to on page 49. For instance in either valley we have decided that the perennial grassy-edged streamlets are of the greatest danger and this is in accord with the prevalence of the anophelines, which normally occupy such streams, being correlated seasonally with the malarial incidence.

Finally, a reference may be made to the contributions of the Tea Estates Medical Officers in Part II (on page 19 *et seq*). There it will be seen how the incidence of malaria in each selected district conforms to our main conclusions, exhibiting also interesting features of other importance. Sir Malcolm Watson's main conclusions (1924), regarding malaria in its relation to the lie of the land in Lakhimpore District, is given in Appendix V (B). Appendix V (C) is an analysis of the data in the two valleys with an attempt at an explanation.

*** The Assam Tea Gardens comprise those in the two great valleys, the Assam (or Brahmaputra), and the Surma.**

† We give in an Appendix V (A) the conclusions as submitted in the Report on the Preliminary Survey.

PART VI.

RECOMMENDATIONS.

We will not here traverse the whole gamut of anti-malarial operations which are on the list—lots of them never would be missed—but rather restrict ourselves to those which appear to us to be the best for the particular conditions which we have found in the Assam Tea Gardens. For those who wish to study all the devices from which a selection can be made, reference is invited to Ross' ' Prevention of Malaria.'

It must be distinctly understood that the recommendations which we will make are the very general ones which are indicated by our very general survey of Assam. We give no others, but that means that they are applicable to every estate. On the other hand, if the principles on which our recommendations have been based be understood, there can be no limit to the ingenuity with which measures can be devised.

It must now be recalled that the main objects of our enquiry were to find out, if possible, if healthy sites on the garden lands are available, to localise anti-malarial operations within the danger zone of human habitations, and to find out which species are causing the damage so that *specific* measures may be instituted to deal with them, and we accordingly submit our recommendations on the basis of our conclusions in these and cognate matters.

Site selection.

If it be decided to plant human habitations on the gardens in as healthy sites as possible, our findings indicate the best which are available. To do that also connotes a knowledge of what sites are dangerous, for they must be avoided as far as possible. Watson in Malaya found that *umbrosus*, a strong flier, was of no economic importance if its ' breeding-place ' was more than a quarter mile away. This species is of low infectivity, and one of high infectivity would probably be dangerous at this distance. A half-mile away from *funestus* breeding-places would probably be economically safe.

We do not subscribe to the view of the mugwumps that one can do nothing on an estate if neighbours will not co-operate. Malaria elsewhere is very local in her operations and in this regard nothing exceptional in Assam has been observed.

It seems too ridiculous that in the past the estate Medical Officer has been so little, if ever, consulted in the matter of site selection. Human habitations, both cooly and European, have just been dumped down anywhere, with the result that millions of rupees have been lost to the tea industry which could have been saved. Perhaps perfectly good health to the cooly and the Babu and the European, with a corresponding gain to their happiness and prosperity and vastly greater profits to the industry, have been sacrificed on the altar of ignorance in making the lay-out of a garden, at any rate as far as human habitations are concerned, subject to the mere whim of a thoughtless person.

Where this has happened, there is now but the alternative, presuming, of course that something is to be done, of sacrificing the cost of such habitations and moving them elsewhere, or of trying to make them healthy *in situ quo*. The comparative cost of attaining the same result must, of course, be calculated in each instance before taking a decision.

For instance, sometimes it appears necessary to cut out tea in order to provide a suitable site, and apart from any humanitarian aspect of the problem, whether it would be more profitable to do this and have healthy coolies at the cost of the tea, or to leave them in an unhealthy site must be calculated by the administration, as it can be exactly for each alternative.

Generally speaking, we have seen above that habitations placed near to great or moderately-sized * active rivers are comparatively healthy, and if along their banks, any place can be found away from permanent streams tributary to the rivers, or away from bhils, malaria would probably be non-existent there.

Another site which is very desirable is on the "pathar-level." By this is meant any wide extent of apparently level paddy land, usually the flood plain of a big river. The conformation of the great valleys of Assam is essentially a series of terraces one above the other and the surface of any terrace is usually called the pathar. But no habitation on the pathar should be placed near any bhil, or to a grassy-edged streamlet, particularly where its course is fairly straight, the water clear, and the bed silty. Saving this, the site may be in the middle of the pathar or on one of the tilas or banks which everywhere are seen arising out of the pathar.

No healthy site can be found anywhere near to a perennial streamlet, even a mile away is productive of a spleen-index of about 20 per cent. If one can find a place far enough from such a streamlet and also far enough from a bhil and near to no hulas especially such as are planted with rice, that place would probably be quite free from malaria.

If these provisos cannot be met, the only thing to do is to get as far as possible from the sources of danger, and complete the work by other anti-malarial operations.

The principle of site selection is involved in the common colonial doctrine of not placing the pioneer's house anywhere near a native bazar, wigwamry or kraal.

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* A moderate-sized stream may be to some extent dangerous if, during the dry season, it leaves pools of water in its bed.

A hospital should be avoided like poison. In this application of the principle one merely protects oneself from mosquitoes most likely to be infected and only runs the risk of infection from others less likely to be infected.

There is no doubt but that the further the cooly lines and bustees are placed from one another the less is the chance of cross infection, but at the same time in a highly endemic area that will not be sufficient to save their inhabitants, while anti-malarial operations will be more difficult. Many medical officers in Assam prefer the coolies to be as scattered as possible, but as long as human beings have to be provided with sanitary surroundings and other amenities of civilisation, they will have to accommodate themselves in aggregations in towns and villages. It would be just as reasonable for Calcutta residents to wish to do their office work on the moors of Scotland. In the event of a healthy site not being found in the lay-out of an estate, it is advisable to have the human habitations as near the centre of the estate as possible so as to afford the greatest subsequent freedom for anti-malarial operations around the site.

If these principles be in future used with intelligence, it may be safely said that a great deal of money and misery will be saved.

Another point in this connection, though of minor importance, should be borne in mind. On most estates on alluvial tracts there are usually here and there some lenticles of a shingly or sandy nature. Such patches are very good sites for lines, for surface water in immediate proximity to the lines is minimal in quantity.

Mosquito control. *

Probably the most important of the various objects of a malaria survey is the indication of the species which are causing the trouble with the economical view of dealing with them alone and these by appropriate measures.

In this survey, we have concluded (*see* also page 14), that *funestus* and *aconitus* are the *universally* important factors in the problem, that *maculatus,* † *umbrosus* and *culicifacies* in certain places must be considered, while *jeyporiensis* occurring here and there cannot be of serious importance to the tea gardens.

Before, however, discussing these species further, we must make one or two general remarks concerning mosquito control.

The first thing is we must always remember to work outwards from an affected centre, so as to get the maximum benefit as soon as possible, and continue doing that till success is attained. In this regard, some remarks have been made above concerning the probable danger distance of the production areas of the species, but the proof of the pudding lies in the eating and no one will wish to do more than necessary.

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* In submitting this section, we wish to say that we do not subscribe altogether to the view expressed in a letter to one of us, 'I wonder if it strikes you what a hopeless proposition it is in Assam to try to eradicate the mosquito—with the unlimited power of Gorgas, one might mitigate it slightly,' but we certainly agree that it would be hopeless to attempt to *eradicate* the mosquito, and needlessly expensive. Sir Ronald Ross has stated mathematically in his Prevention of Malaria how merely a slight reduction of mosquitoes has a more than proportionate effect in the reduction of malaria.

† The evidence which we have collected against this species is more circumstantial than direct. More research is required in connection with it.

Another point in general about mosquito-control is that it is better to tackle the problem in the mosquito 'breeding places,' that is during the fly's larval state when measures can be restricted to a limited area, from which the larvæ cannot escape. In the adult stage they have, of course, much more scope for eluding us. Perhaps the adult can fly, as reported in America, 30 miles.

Now, while we will discuss here the control of all of the species mentioned, we would as a general principle recommend that all anti-malarial work undertaken on estates should be focussed first on the extermination of *funestus* and *aconitus*. Even where the other species on the black list are to be found and may be causing trouble, these two species are almost certainly to be found, and we feel confident that if a campaign against them be initiated and nothing more be done, then the malaria-rate in the tea gardens will largely disappear.

With this in view let us recall where *funestus* and *aconitus* are to be found so that we may deliver them a shrewd blow and not waste our financial and muscular energy on anything else, and then let us discuss the most appropriate measures for dealing with them.

funestus and *aconitus* are found in great numbers in the grassy edges of streams (Fig. 12), whether perennial or non-perennial, though the grade of a stream seems to have an important bearing on their numbers. Thus on the so-called pathar-level they are not so common: however, one may often find them in swamps, bhils of still water, tanks, ponds, and such like places. Perennial streams are, we have seen, very dangerous and probably in general the places where water lies the longest are most so and should be attended to first.

As perennial streams are so important, it must be remembered that they form but an element in certain parts of the general physiography of the country, such as hulas, jans, etc., and therefore any recommendations made for the treatment of the streams may have to be modified to suit the local conditions.

We recommend the following treatment of these streams. The first thing to do is to canalise them,* in obedience to the general principle of water tidiness: It is principally in the little bays and pockets formed by the falling in of the banks that the species are found. This canalisation should be a capital charge and maintenance an annually recurring one (*see* Appendix VI). For the latter (and for other purposes) a sardar, or sardars, and a gang of coolies should be organised, the Sanitary Squad.

When canalising a stream one should follow the direction which it has carved out for itself in nature, in obedience to the equilibration of forces represented in the structure of the earth and the flow of the water in the stream. The cutting-off of loops is silly from a dynamical point of view and when one remembers that the lower the gradient of a stream the less is it to the liking of *funestus*, then the loop elimination is undesirable also from a biological point of view.

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* We presume that subsoil drainage cannot be contemplated.

Then another operation than canalisation is necessary, that is the keeping of the banks free from grass at the edges of the water in which places the species love to lurk. This would demand the constant attention of the Sanitary Squad and the job might seem to be an enormous one especially during the rains under the luxuriant conditions of tropical life. But it can be effected as it was in another luxuriant country, Panama, that is the grassy edges and banks are sprayed with oil from a knapsack sprayer and the banks then set fire to. After a few such operations the earth becomes covered by a thick-felted mass on which grass will not grow.

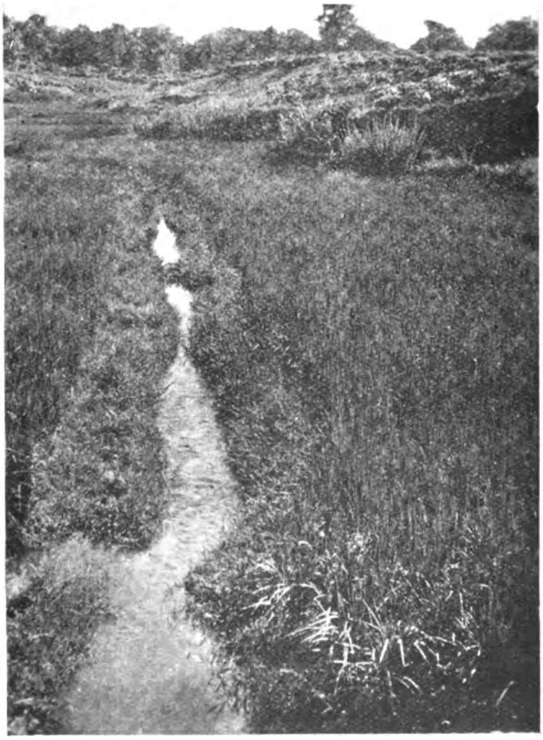

FIG. 12.—*funestus* and *aconitus* are found in great numbers in the grassy edges of streams.

Oiling the grassy-edged streams without the burning process might be tried, though it is doubtful whether it would be successful owing to the film being rapidly washed away to the centre of the stream and for other reasons. Good reports have already been published of the efficacy of *Paris Green* as a larvacide and when opportunity occurs to us an experiment will be carried out on a *funestus* stream to test it. This substance when dusted on to the surface of water is said to delude larvæ into swallowing it in mistake for food whereby they are poisoned.

Canalisation and dealing with the grassy banks of the stream can be dealt with, as it has been at Lumding on the Assam Railway, by using concrete or brickwork inverts. Such work is very satisfactory though expensive, and should not be indulged in unless under special circumstances as in towns.

We feel, however, that all these methods of dealing with the grassy edges of a stream after it has been canalised are unnecessary and inefficient when nature has provided us with an easy and inexpensive means of eradicating the pests. If merely a strip of jungle be grown along the banks of these streams, there will be no more *funestus*. What could be better, or cheaper? We do not, let it be noted, ask that the whole of a tea estate be let go into jungle, but only little strips along the canalised streams. Of course, we would not recommend it to those who like to splash money about, for the entertainment would be most boring to them. We rather invoke the physician who knows what a faithful handmaiden is *Vis medicatrix naturæ*.

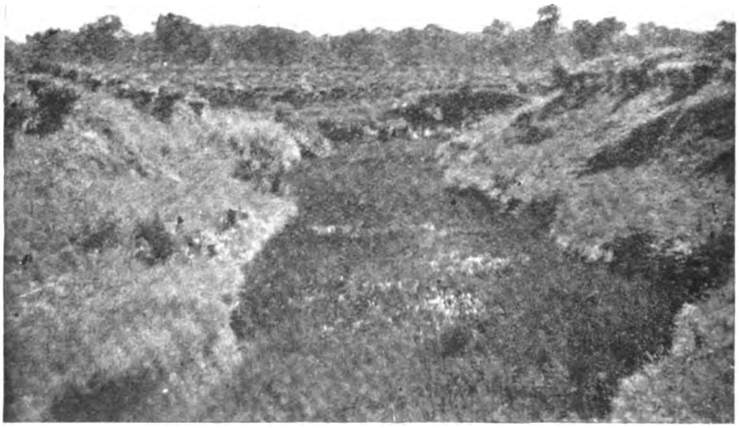

FIG. 13.—It is doubtful if a hula which runs dry for many months in the year is of much importance.

Seldom is it that any débris or grassy patches are seen in the middle of these streamlets. If such are found, they must be attended to by the Sanitary Squad, whose watchword is *water-tidy*.

Now we must refer to certain places where these grassy streams are involved, for instance, the hulas, jans, swamps, bhils, etc.

Hulas. It is doubtful whether a hula which runs dry for many months in the year is of very much importance (Fig. 13). One would be able to dissociate their influence in a locality from that of simple perennial streams by treating the latter only just as one sometimes diagnoses malaria from other *pathologia* by the reaction to quinine.

Nevertheless, the non-perennial streamlets in hulas often teem with *funestus*, and to deal with these one can do as advised above.

Hulas, as described previously, are usually planted with small patches of paddy, and to irrigate it water may be taken from the head of the hula in irrigating channels. These must be treated in the same way as advised for the streamlets. To have an irrigation channel covered in by a riband of jungle will not affect its utility in any way.

As far as the paddy itself is concerned, we will defer a consideration of this to a later page.

Jans, bhils and *khals*, from which there is a regular flow of water: these, as seen above, are dangerous and appear to be so in proportion to the number of streamlets in them.

It must be recalled that such places are old river beds whose function has largely ceased, other more active river beds at a lower level having taken on the duties. That being so, in such places there will always be found a fall for drainage, if we wish to utilise it. And drainage must be considered in relation to the treatment of streamlets in jans and bhils, for very often these streamlets emerge but gradually from a great swamp and their canalisation would be impossible without first reducing by drainage the general area of surface water.

In the paper read by one of us (C. S.) before the Assam branch of the B. M. A. (1925) was the following :—Drainage, strange as it may seem, does not appear on the schedule of 'measures of general application'. Three of the malaria-carriers prevalent in Assam—*A. maculatus, funestus* and *aconitus*—are stream breeders, so what one does by confining collections of water into regular channels is to increase the pace or flow of the water and therefore make it easier for *maculatus, funestus* and *aconitus* to breed. Watson in 1911 demonstrated this ; he drained his swamps, whereupon *maculatus*—which is not a swamp breeder—obtained a habitat to its liking and malaria was increased by the measures taken.* And there is no *á priori* reason to suppose that what happened in Malaya would not happen in Assam, or an even worse thing, because here the species *funestus* is so prolific, while it is altogether absent in Malaya. There is no doubt whatever that it is chiefly in the drains dug by the native cultivators for irrigating purpose that *funestus* and *aconitus* are principally found.

'There is, however, the possibility that the reduction of the area of water in a bhil may countervail the factor just mentioned and the sum total of *funestus* output be reduced. Drainage systems are now being used with this idea by Watson to control *maculatus* and *funestus* in the Doom Dooma gardens.

From evidence obtained since writing this, we have now come to the conclusion that the general reduction of water in a jan by drainage certainly does countervail the advantage of having swamp as opposed to running water, the countervailing factor probably being the reduction of the number of streamlets in the bhil. For an example we refer to p. 66 Ba 3 above. Moreover, it is better, other things being equal, that land should be available for cultivation than under deep swamp.

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* 'Incidentally this example may dispose of the ridiculous hypothesis that a species of mosquito driven out of one type of habitat will establish itself in another.'

In this connection Watson (1924) has adduced some potent examples showing the probability that drainage and drying of these sorts of places is likely to improve matters considerably.

In general, then, we may take it that whatever the spleen-index in a locality may be, it will be reduced, first by drainage which acts probably by lessening the number of streamlets, and secondly by canalising and otherwise treating the streamlets.

Drainage (Fig. 14). Too many drains should not be dug in any given area. In one garden we have seen so many deep and wide drains that there was the merest trickle of water in each, though the total volume in the collecting channel was considerable. That state of affairs is dangerous. Dr. Hugh-Smith tells us of one of his estates where malaria was 'only reduced by filling in a number of drains.'

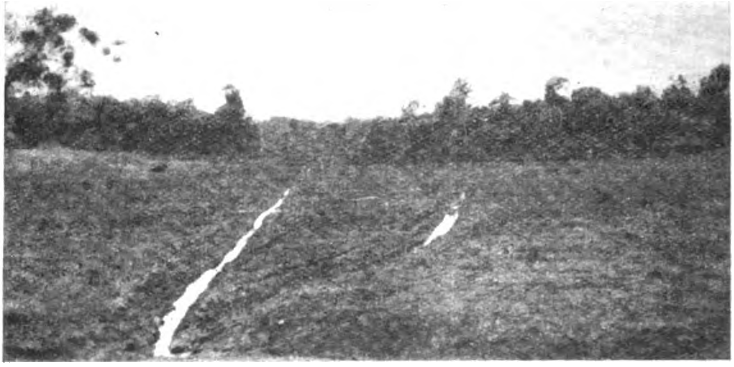

FIG. 14.—Drainage.

Drains should, if possible, have a grade of between 1 in 250/400.

Swamps. Certain places are swampy without being 'jans' or 'bhils.' Sometimes they are due to a constant seepage from hills behind, i.e., hill-foot swamps. From them, too, streamlets gradually emerge (Fig. 15,) and eventually find a channel for their escape. As swamps, the same principles as proposed for jans must be applied, but the technique of the drainage is different, for here the seepage must be intercepted by deep hill-foot drains.

Drains have been proposed for jans and for such swamps as described in the last paragraph. Drains for carrying storm-water only, as in villages, are also sometimes inhabitated by *funestus*. The treatment suggested for them has already been mentioned, viz., reinforced concrete inverts (Fig. 16).

Bhils (in the narrow sense that there is no outflow from them but merely act as sumps for storm-water). We have seen that generally speaking, bhils are not responsible for a high malaria-rate: nevertheless, they may be dealt with. In the first place they, like any of the less 'advanced' bhils mentioned above, may be drained; or, as the water in them is mainly sumpage from the local rainfall, this may be precluded from entering, if not desired, by digging a catchment drain all round the bhil and its feeders and leading the drains off to some natural drainage channel.

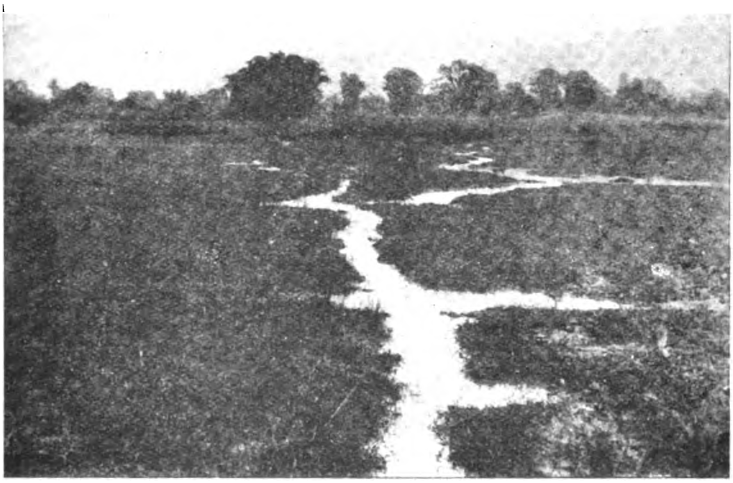

FIG. 15.—Swamps due to seepage from hills, streamlets emerge.

If the collection of water is useful, it has been advised by one of us on a group of gardens in South Sylhet, of which he is in charge, to dig a series of contour terraces in the bhil, which will cut out the dangerous shelving bank. One of the duties of the Sanitary Squad would be to maintain the banks more or less steep. The bhil could thus be used as a tank and convenient pukka access to it devised.

The *maculatus* side of the problem, as stated above in many places, we do not consider to be of much general importance to the industry, though where it is found it should certainly be feared and dealt with efficiently. Its habits have been indicated above (*see* Introduction, page 11). Watson found it in spring water at the foot of the banks at the side of jans. We found it in hulas, in spring water, and we suspect it to be the cause of certain high endemicity of malaria in some of the high tila gardens of Cachar (Fig. 17). It certainly is so in gardens planted on the rocky foot-hills of the Shillong spur.

Hulas have already been mentioned as far as their streams and water channels are concerned, and are also included in the general remark as to the advisability of drainage in general as a means of reducing the *funestus* danger. The latter suggestion affects the *maculatus* problem as well. Although, as mentioned above, drainage generally encourages *maculatus* and a swamp, *vis-a-vis maculatus*, is better for health, yet, under certain conditions, that is not true, e.g., when the swampy ground is rocky or sandy, in such places it being better to drain. However, in view of the probable benefit of drainage in reducing the *funestus* danger and the doubtfulness of the *maculatus* danger, we would drain hulas. Nevertheless, we can devise the drainage to be of most benefit, in case *maculatus* be present. The drains must be at the hill-foot along the contour to intercept the seepage from the banks and tilas above. The drainage channels for *funestus* need not be at the hill-foot, but they *may* be there and so hill-foot drainage kills two birds with one stone. Moreover, a third bird comes into the brown under certain contingencies, for a hill-foot drain is the most suitable as an irrigation channel for rice cultivation and water may be taken from the head of the hula for this purpose and this also may have the effect of flushing out any *maculatus* which are present. To crown all the riband of jungle, which we propose should be grown over the hula irrigation channels to deal with *funestus*, will also have the same effect of smothering *maculatus*.

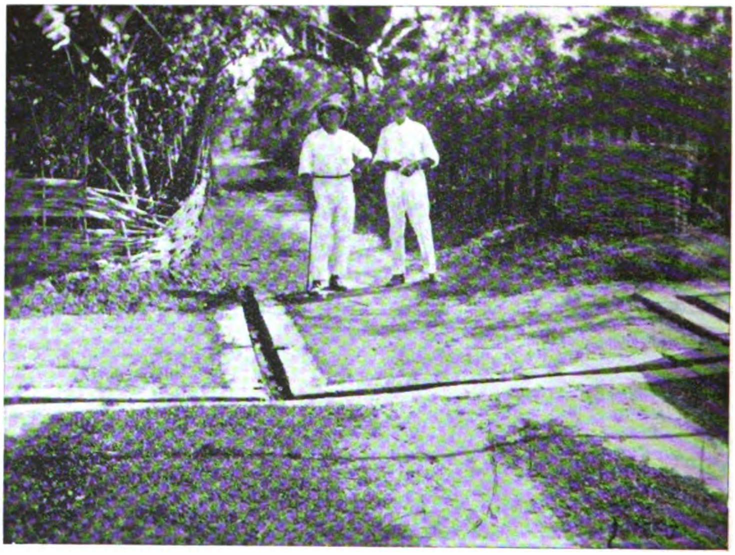

FIG. 16.—The treatment suggested .... concrete inverts.

maculatus would probably be more difficult to deal with in the stony streams emerging from the Shillong hills (Fig. 20). Springs of water oozing from the hills might be dealt with by drainage and the drainage *cum* jungle method, though the pools of river water left behind on the bed of rivers would perhaps best be dealt with by oiling (Fig. 21): the only objection to which is its constantly recurring nature and never ending expense. Masonry works would be effective, but are expensive (Fig. 18). Small rocky streamlets might at small expense be dammed across and silted up in a series of terraces (Fig. 19).

The *culicifacies* danger. This is exemplified by the stream at Lumding where the species was found in pools with *maculatus* in the bed of the silty stream. At Lumding, the medical officers tried the flushing system by damming up the stream to a certain level and then 'letting it go', but eventually reverted to oiling the pools in which the species was found (Fig. 21). At Lumding, too, a big dhoby tank was reported to have carried a large number of this species and it was eventually filled in.

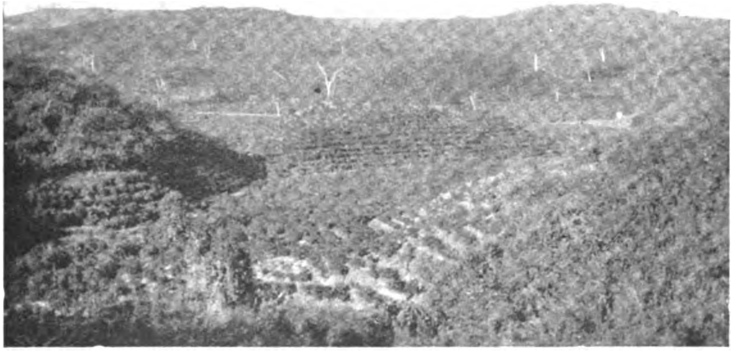

FIG. 17.—The high tila gardens of Cachar.

With regard to tanks in general, it may be said that we never found a single specimen of *culicifacies* in any tank in the Assam tea district, so the danger is not great. Nevertheless, we obtained *aconitus* and *funestus* in them and as they are a necessity, one must be prepared to make the best of them. Generally speaking, they must be treated for the eradication of *funestus* by destroying the grass and weeds on their edges by the methods advised above for streamlets. And a further measure can be advised: the small fish found in tanks eat mosquito larvæ, so they must not be caught by coolies using fine mesh nets: on the other hand the more big fish that are caught the better, as they eat large numbers of little fish.

The *umbrosus* problem. *umbrosus* may be reckoned a potential danger whereever there is undrained jungle. The species will not live in running water, but only in depressions on 'flat' land and in borrow pits or in bhils or swamps in the jungle. Where these conditions exist there will be danger, but with running water in jungle there will be none. Consequently every situation must be judged on its merits. We have seen estates surrounded by jungle with a very low spleen-index, and habitations with a high spleen-index similarly situated. When the jungle has been correctly incriminated, it should not be cut down, for then *funestus* will make its appearance. The jungle breeding-places should be filled in or drained. Thus bhils can be drained or borrow pits filled up; *umbrosus* cannot live in a functioning drain.

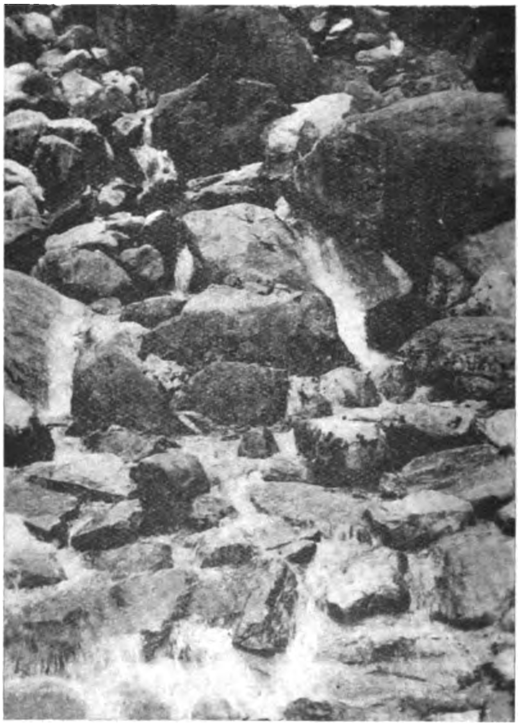

FIG. 18.—Masonry works would be effective but expensive.

Above, we suggested covering up streamlets with ribands of jungle, and this, it is now seen, is perfectly consistent with what is advised for dealing with *umbrosus*.

We will later on advise that paddy or other wet cultivation be put a stop to altogether in hulas, and if that be done the hulas should be drained, if that have not been already done, and then the whole place allowed to revert to jungle (Fig. 22).

While drained jungle is probably the safest environment in Assam to live in,* it must be remembered that there can be but little objection to cutting it down in those tracts of country which are, when cleared, comparatively non-malarious, for instance, the so-called pathar-level. Perennial streamlets on the pathar-level seem to be just as dangerous as elsewhere, but they can be dealt with effectively as described above for *funestus.*

FIG. 19.—Small rocky streamlets might be dammed across and silted up in a series of terraces.

We must now make some remarks on more general methods of mosquito control.

The first concerns water tidiness. When any species breeding-place is "tidied up," it is rendered less suitable for the life of the mosquito because its protection and food is removed. In pools and ditches of slowly running water, this implies the clearing out of as much débris and foreign matter as possible, also the oiling and burning of all plant life; in streamlets, it implies the keeping of the banks and edges clear of vegetation or facing them with concrete or steps, and 'training' or deepening the water channel to such an extent that plants cannot grow.

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* Mr. Descennes, Superintending Engineer, Assam, tells us his experience has been that opening up jungle leads to ill-health. On the other hand, in the Assam Administration Report for 1876-77, p. 149, was the following: 'The comparatively disproportionate area occupied by swamps and dense jungle growth . . . . the removing of jungle in this recently from the station of Tura has resulted in a marked general improvement of health.'

Water tidiness has been the *raison d'etre* of the drains put in at Lumding, where the old earth drains have been lined with a concrete invert (Fig. 23) constructed of reinforceed concrete and deep enough to carry off the normal drainage and the banks above the drains shaped and smoothed down and turved.

Secondly, *subsoil drainage and filling.* Subsoil drainage will not be discussed here as in Assam it is not "practical politics". Another general measure is filling, but it is expensive. The tank at Lumding was filled. The principle is the same as subsoil drainage, that is, the water is kept underground. In Orissa, we have seen shallow natural nullahs, which are prolific breeding-places of certain malaria-carrying anophelines, filled in by the natives to level up the land for rice cultivation; an instance of economics and sanitation going hand in hand.

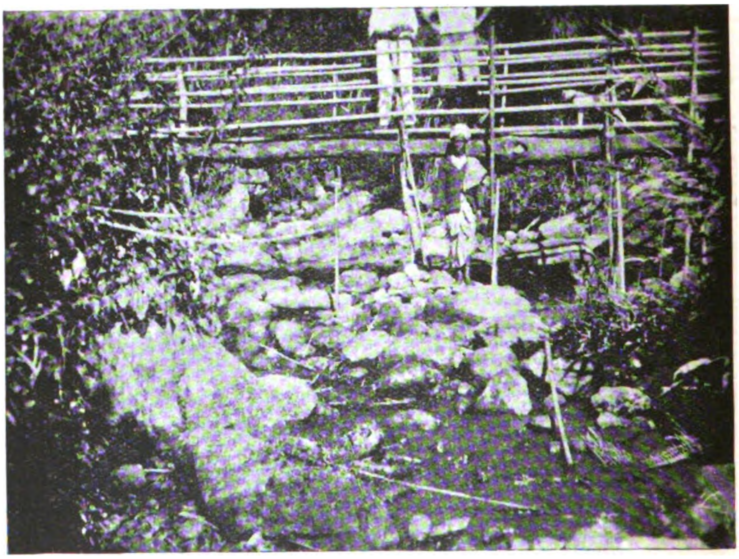

FIG. 20.—*Maculatus* would probably be more difficult to deal with in the stormy streams emerging from the Shillong hills.

Thirdly *flooding.* More might be made of this measure. The practice is to throw a dam across a valley, the effect being to create a large body of deep comparatively still water (Fig. 24), where swamps or running streams existed before. Danger may ensue at a later stage when silting takes place to such an extent that there is a mass of floating water plants. Moreover, seepage below the dam may be a source of danger. At a still later stage, the embankment may hold up a solid mass of silt over which the original flow is distributed if it be not retained beneath the surface (C. S. 1925).

Fourthly, *good conservancy.* This has its advocates as the sole means necessary for averting malaria, but it was shown above that a Jainti village on the Shillong foot-hills, which was spotlessly clean (Fig. 25), was highly infected with malaria. Mosquitoes would, of course, not find so much protection in a habitation kept scrupulously clean, but it is obviously not a measure to be of more than accessory value in dealing with a hyperendemic area.

Fifthly, *paddy cultivation.* On a previous occasion one of us after excluding the paddy of the pathar-level from consideration said that one was confronted by a problem something like a jigsaw puzzle complicated by the fact that oiling, jungle growing and flooding were incompatible with rice growing. At the same time he wrote 'further study of the detailed observations which have only recently been concluded may of course oblige us to modify the opinions expressed.'

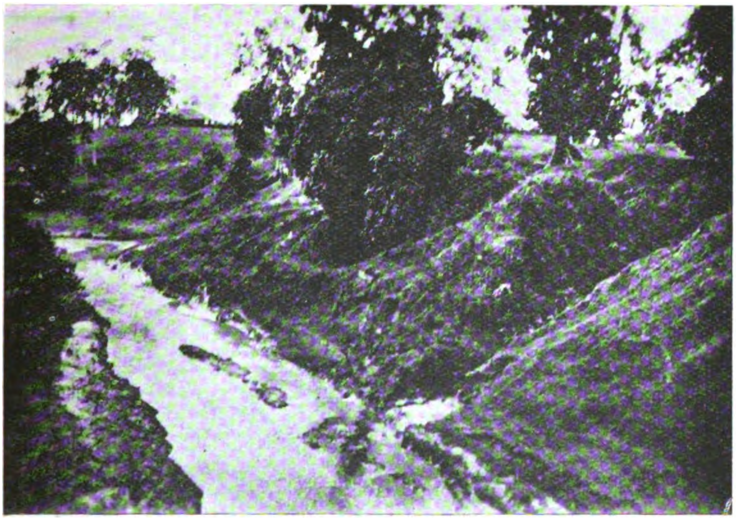

Fig. 21.—The pools of river water left behind on the beds of river perhaps best dealt with by oiling.

What we have now written below on the subject is a modification of the views then expressed.

In the first place, the paddy of the pathar-level cannot be altogether excluded from consideration. Certain perennial streamlets which course over it must be attended to and the considerable tracts of paddy fields themselves remain for the discussion below.

In the second place, we are inclined now to regard the streamlets and irrigating channels, particularly when perennial, as being the particularly noxious elements of the paddy field tracts when they are not on the so-called pathar-level. These tracts of paddy lie on the flood plains of the tributaries of the great rivers; the plains constitute the pathar and as the tributaries become smaller, the flood plains do so also and finally constitute only the beds of hulas.

As we ascend the flood plains (Fig. 26) from the great river flood plains or pathars, we find the streamlets becoming more and more dangerous, but it is problematical to what extent do the paddy fields which are irrigated by those streams. Probably as we go higher, and higher the paddy fields, like the streamlets, become more dangerous, though it is never easy to find *funestus* in them.

It is obvious that the great plains of rice cultivation cannot be given up and luckily, that would in no case be necessary if the streamlets be dealt with, which would not interfere in any way with the cultivation.

FIG. 22.—The whole place allowed to revert to jungle.

On the other hand, at the top end of the scale we have the little hulas planted with rice. The rice in them, probably, is of some danger apart from the irrigating streamlets and as from the economic point of view the hula rice must be negligible in value, we ask that it be given up. We appreciate the sentimental value attached to it, but second Mr. Crawford when he says some discipline there must be on every garden in the interests of health.

The passion for rice growing seems to be something of a fetish for in 'Castes and Tribes of India' published by the Tea Districts Labour Association, one reads the following passage: 'Facilities for the acquisition of land have in the past been held out as decided attractions to estates which are in the position to grant holdings, either free or at a nominal rent, to the permanent labour force. This factor still, undoubtedly, carries great weight with potential emigrants but at the same time many instances could be cited, particularly in Upper Assam where khet land has never been available, yet labour continues to flock to the estates in all seasons. It might be argued that such concerns are in this fortunate position owing to their strong connection in the recruiting districts, but when we consider the favourable economic conditions that have obtained in the districts during the last three seasons and the fact that sirdars of other estates, having the attraction of khet land to offer, have also been endeavouring to recruit, but with comparatively indifferent success, we are forced to the conclusion that the attractiveness of an estate lies, not so much in the offer of material amenities but a feeling of confidence and faith in the management, in brief, the personality of the manager.'

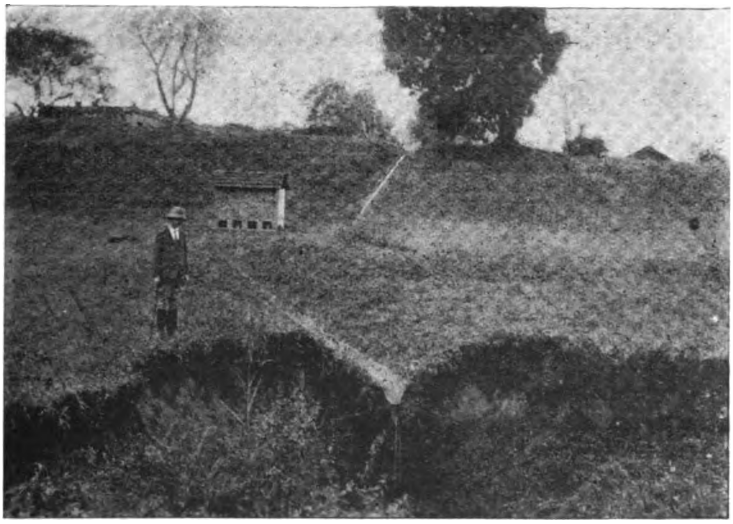

FIG. 23.—The old earth drains lined by concrete.

We have heard everywhere the opinion expressed that it would be absolutely impossible to try to run an estate if the cooly has his rice land taken away. In spite of that the next day perhaps that so and so has stopped his rice cultivation. The trumpets have blown, but the walls of Jericko are not even shaken.

We are quite convinced that the main source of the malaria in the tea gardens is in the perennial streamlets, and we now merely dismiss the question of paddy cultivation (except that in hulas) rather lightly as we think that there will be quite enough work to do for some time on all the estates in dealing with the streamlets.

We offer the paddy fields to the irresolute squad of managers as a sop to their sentiments, while they reduce their malaria-rate considerably by dealing with the streamlets. When that has been accomplished, the position of the paddy fields in the residual malaria can be reconsidered.

In the meantime we cite what one of us has already said previously.

'The following suggestion might be made :—

(1) That coolies be given their dhan-khet at some distance from the lines and the intervening land not rice cultivated, and the lines concentrated so as to reduce the total non-cultivated area. I am told that the cooly likes to see his rice growing under his very nose, but on the other hand a prominent planter of the Jorhat District, in response to a demand from me that the planter himself should put us suggestions as to how the difficulty should be met, informed me that coolies would be quite content to have their khets some distance away. This view seems borne out by the fact that coolies will sometimes leave their khets and bustees for long periods to work on estates, only returning for the harvest.

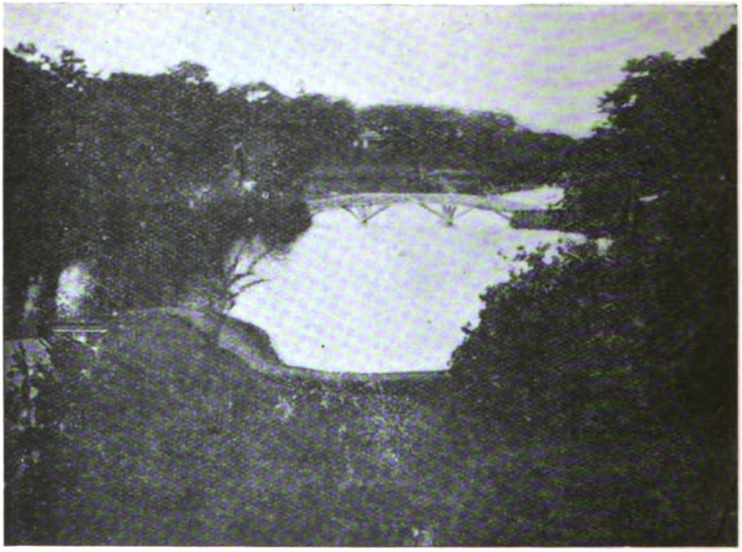

FIG. 24.—Create a large body of deep water.

If the lines then be concentrated on one side and dhan-khet parcelled out to the coolies as far away as possible, the intervening area can be treated to the special measures which are necessary, either flooding, jungle growing, or draining and oiling.

(2) If that suggestion be not liked, another to be submitted is based on our knowledge that it is the small hulas which are particularly dangerous ; therefore, I would ask that the cultivation of small hulas only be given up and if that be not admitted, that at least very dangerous irrigating channels may be dealt with in accordance with the general principles laid down, though I am afraid the results would be but meagre' (C. S., 1925).

And not only do we offer the paddy fields, as distinct from the irrigating channels, as a sacrifice on the altar of mosquito control, but we would urge that the primary effort should be to deal with the *funestus* problem as apposed to the *maculatus*, *culicifacies* or *umbrosus* danger. *funestus* is the keystone to the arch and if that be knocked out, the bridge will collapse.

Measures aiming at reducing the chances of the mosquito becoming infected.

(1) *Segregation of infected persons.* It is, of course, better that all infected persons should be gathered into one place than that each should be the centre of an infective area. That place should be the hospital, which, of course, provides other advantages than that. Adults with fever often have the peripheral circulation infected with the plasmodia, if only for a short time; children, on the other hand, as numerous observers have pointed out, are the chief sources of infecting the mosquito, having gametocytes in the peripheral circulation comparatively often. Christophers and Khazan Chand (*see* reference) found the parasite in 80 per cent of the children they examined at the close of the malaria epidemic season (at Nalbari in November): we did not find anything like so high a rate but our results were obtained from films taken all through the year.

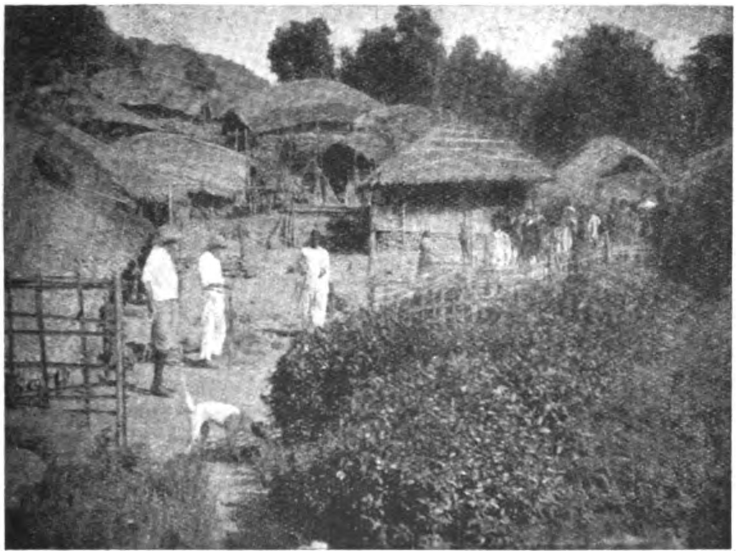

FIG. 25.—A Jainti village spotlessly clean.

Now it is notorious that coolies in the Surma Valley generally have established the tradition of not going to hospital when ill. Hospitals are about as much use to them as bath-rooms in the shearers' huts of Queensland sheep stations, where at least the bath-rooms are used for the storing of potatoes. Whether it would be possible to break down that cooly tradition is a question not difficult to answer. Perhaps even in the Assam Valley it would be possible to get more of the malaria-stricken coolies into the hospitals than is usually accomplished. A lofty ward jack-roofed and cowled is not likely to be unduly hot, but in any case sick coolies would probably benefit generally by having punkhas (electric or otherwise) in the ward and a hospital so fitted would doubtless be more attractive. With regard to the children, the fact that very often there is no apparent illness and the child may be running about while his blood be invaded by gametocytes makes matters more difficult and there appears to be nothing for it but to examine a film from every child and look for the parasites under the microscope.

FIG. 26.—As we ascend the flood plains.

(2) *Screening.* Having got the coolies into hospital, it should be screened (*see* below). If he be not under a screen every cooly in hospital with malaria should be put under a mosquito-net from dusk.

(3) *Quinine to sterilise the blood.*—The above measures in themselves would suffice to reduce the malaria infection rate considerably, but apart from them the blood of the infected persons should be sterilised by quinine. In his paper to the Assam Branch of the B. M. A. (March 1925), one of us (C. S.) said: 'The carriers from whom the mosquito mainly becomes infected are the children and every effort should be made to treat them with quinine to prevent them infecting the mosquito. Parasite parades might be held once a week using Knowles' and Das Gupta's (1924) excellent thick film method for discovering the parasite. Quinine unfortunately has many limitations of which the outward symbol is the excessive malarial incidence of the estates. It is to be hoped the chemists will soon discover something as effective for the malarial plasmodium as trypan-blue for ***Piroplasma canis.***' Since writing the above, the opinion of many Assam medical officers has transpired, to the effect that it would be cheaper to dose all children indiscriminately rather than to pay for the looking of the parasite, but the choice is only an actuarial

matter. Colonel Clemesha has informed us that he has had very good results from using this method.

Measures of protection of the individual from the bite of infected mosquitoes.

(1) For the European, some protection is afforded by the use of mosquito boots and the European in Assam is to be congratulated on his general attention to such matters; but this does not protect other parts than the ankles. For this reason, the use of mosquito-proofed houses or at any rate proofed dining-room and lounge is to be recommended. And it is probably more important than anything else that clubs should be protected. Nobody who has not lived in a screened house can appreciate its comfort. One objection that is often urged against it, is that it is impossibly stifling in the hot weather. Certainly that is so if the screening be fixed, but if made to be opened up from sunrise to sundown, one's experience is that it does not make the air appreciably hotter. This measure is, of course, only applicable to the higher staff of the estates.

'As for the labour it might repay the companies to give it cheap and strong bed nets, starting with those who would apply for them. Everyone knows how the cooly will get under a net except for his feet, but even then his chance of infection is much lessened (and his feet ought to be horny enough to look after themselves).'

Mr. Hobson, M.L.C., of Bertholl, told us that as many as 50 per cent of his coolies had nets, and a wonderful number of children were born there (whether *post hoc propter hoc* he did not give an opinion).

(2) *Airy houses*, etc. *Chang* bungalows for the staff are the best type as there is more air about them and about. The design in view should be primarily the obtaining as much air and to catch as much of the prevailing winds as possible; everything should be subordinated to this idea, like ladies dress nowadays; windows and doors should be large and many; dark cosy corners avoided like the plague; fabrics of all sorts torn down and sent home to the castles in Scotland or Spain, and every tree which is in the way should be cut down ruthlessly, even if it was planted by the Prince of Wales. One point apart from air which should be mentioned is the desirability of having the walls and ceilings of the rooms painted white: lizards, too, like them white, so that they can see their mosquito prey: another advantage.

With regard to habitations in general, we suggest that a competent architect well cognisant of Assam conditions be commissioned to draw up a series of standard designs for different grades of the estate staff: the doctor should have the best. The result would probably be pleasing to the eye and useful as an anti-malarial work.

To crown all, electric punkahs are very much to be desired as a means of preventing mosquitoes biting. Coolies to drag a pull-punkah appear to be unprocurable nowadays.

Intrinsic protection of the individual.

(1) *Quinine prophylaxis.* One must now consider whether that famous prophylactic measure—quinine administration—is useful, i.e., would quinine given

to a whole labour force prevent it becoming infected by infected mosquitoes. The problem has been analysed by Dr. Hermitte in an address to the Assam branch of the British Medical Association. He came to the conclusion that quinine prophylaxis is efficacious. He recommended two doses a night to ensure perfect results, as quinine is excreted 8 hours after its ingestion but thought that only one dose would do a lot of good. On the other hand, Dr. Forsyth in his carefully-controlled experiment at Kacharigaon, found that with such a system as one would use in ordinary practice it produced no good results.

Hermitte did not mention the very carefully-conducted experiments conducted by Army officers at Salonika during the war. We think that if British army officers with highly disciplined troops under war conditions came to an adverse conclusion in the matter, their opinion should be regarded as final.

Watson's opinion is that for prophylaxis no amount of quinine is of any avail to stamp out the disease, and that malaria will not be eradicated unless some medicine vastly more effective than quinine is discovered (or mosquitoes are destroyed).

In general, therefore, it may be concluded that quinine prophylaxis * does not offer sufficient inducement to us to trust to it rather than to, say, mosquito control.

(2) *Bonification*, the high-sounding title given to the process of improving in any way the lot of the labourer : much has been written and said on this subject in its relation to public health and particularly malaria, especially by communists who have spoken with their tongue in their cheek. There is a germ of truth in the contention that prosperous persons do not suffer much from malaria. Suffice it here to say that while epidemic malaria does not appear to be quite so serious under a bonifying regimen, endemic malaria is not affected.†

(3) *Immunity*. Passive immunity cannot be induced in malaria, at any rate it is not a practicable proposition, while the immunity or 'saltedness' of persons who have been brought up in hyperendemic regions is the end-product of conditions which it is now the effort of everyone on tea estates to try to avoid.

It is, of course, common knowledge that salted coolies, the so-called 'good jungly coolies' are the best labour to recruit because of their comparative immunity, but it must be remembered that their children, if born on malarious estates, will have to go through the same mill as their parents and many will be crushed in the process.

It would certainly seem nevertheless a better financial proposition to bring up such children on the estates than import 'yellow-bellies' who, like the children, must become immune through suffering. But the jungly folk cannot be got in sufficient numbers, at any rate at present, so unless estates can be made healthy, the others must be entertained to the hospitality of the industry till they have shaken off the evil humours.

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* This must not be confused with the attempt to sterilise the blood of infected persons (page 125).

† The matter will be the subject of more extended notice on a report by us on the Dooars now being prepared.

Accessory measures.

We here repeat the gist of what one of us has written elsewhere (C. S., 1925).

There are certain measures which are quite ineffective in themselves as anti-malarial agencies, but which, nevertheless, are quite important in the general campaign.

(1) *Education and propaganda,* amongst the directors, the shareholders, and the coolies is required.

The cooly on some gardens is treated to a cinema show and perhaps he could be 'got at' in this way by suitable propaganda.

As for the managers and directors, they have only to be shown reasonably and lucidly what has been accomplished elsewhere to be ready to furnish the sinews of war. In fact usually very poor arguments seem to be effective in making them pay up. They are, of course, nervous lest experiments should be conducted at their expense and prove costly failures, for unfortunately money spent on the prevention of malaria has not always been repaid. One way of re-assuring them would probably be, for the Assam branch of the British Medical Association, to proceed by resolution from time to time on agreed upon measures which may be confidently recommended to the public. One resolution, for instance, might be that in the future lay-out of a garden the medical officer should be consulted; the present lay-out of some gardens showing the necessity for such a proposal: the directorates would doubtless feel that such a proceeding protects them from faddists. The instance might be cited of how the executive in Malaya was let down by a highly-placed medical officer who thinking *qua* the lady-novelists, that mangrove forest was deadly got money for cutting it down: he acted in quite good faith, but could not imagine that such a measure converts a healthy place into an unhealthy one.

We therefore have to put a good case up to the people who provide the money. Watson has said that most of his life he had spent in fighting the men who were preventing him from fighting the mosquito, so now in Assam it is to be hoped that the directors will immediately vote a large sum of money for propaganda among themselves.

(2) *Financing anti-malarial measures.* We are very strongly of the opinion, expressed also by Watson,* that the cost of anti-malarial work should not affect the commission of the staff of an estate. If it be made to do so, the practice will act like a dead-weight on all the efforts of those who are trying to do some good.

(3) *Technical education of the Doctor Babu.* It is an excellent idea that prompts Dr. Hermitte with the sanction of his directors, Messrs. Williamson,

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* Watson says: 'From experience of efforts at what proves to be false economy in the end, I can foresee some danger to the work, if it affects the commission paid to the staff of the gardens. There are, of course, arguments on the other side. The main thing at present, however, is to get the labour healthier; once that has been done, and the staff are fully cognizant of the methods of controlling malaria, then they can attempt to cut down expenses to a minimum. My strong recommendation is, therefore, that the cost of this sanitary work be paid from capital, or from some reserve fund, and that it does not reduce the commission paid to the staff, who after all look on commission as part of their wages.'

Magor & Co., Ltd., to send his Doctor Babus in a never-ending stream to the School of Tropical Medicine in Calcutta. Every Doctor Babu ought to have a microscope and know how to use it. If he went to the School of Tropical Medicine, he would learn microscopical technique and how to identify mosquitoes and parasites The School Short-course is admirably adapted to meet the needs of the tea estate Doctor Babus.

(4) *Research*. This must of necessity be largely left to institutions equipped for the purpose. One enquiry suggested above was the search for more effective chemical compounds than quinine for clearing the parasite out of the blood. The School of Tropical Medicine fulfils such a function and as it is largely dependent on public contributions for its ever increasing activities, it is to be hoped that these will not be stinted. In this connection we may include an appeal for better statistical material to be kept on the gardens for future guidance of medical workers. Under 'the Malaria Indices' in the Introduction of this report we have drawn particular attention to the advantages from the statistical point of view of extending the scope of maternity benefits. Also, for instance, a card index should certainly be kept for each cooly recruited, stating whether his spleen was enlarged, and if so by how much, on his arrival on the estates, not to mention other medical details.

(5) Recruiting of susceptible coolies should be carried out mainly in the cold weather: jungly coolies can be recruited at any season. The object of this suggestion is to reduce the epidemic rate among those who are bound to get fever sooner or later, and so minimise the dislocation of work on the estates. Moreover, there is some evidence to show that the greater the epidemic rate the more virulent the cases.

PART VII.

CONCLUSION.

We have now reviewed in sufficient detail the various preventive operations which we think applicable, and should be applied, to Assam conditions.

The economic loss to the great tea industry from death, sickness, and defection of good-will, owing to malaria, must be very great, quite apart from the humanitarian side of the matter.

It is therefore, as stated above, very remarkable that so little effort has up to date been made to improve matters,* when practically every other country in the world, especially the plantation countries, have, either through their Government or private enterprise, done so much. Probably the reason lies in the fact that Sir Ronald Ross made his great discovery here.

It is to be hoped that now the proposals we have made will not be thought to be so complicated or expensive as to preclude any idea of taking them up. They, roughly speaking, may be summed up in two words, drainage and jungle growing. As for drainage, the estates pay out many times more for that of the tea land than ever would be involved by our scheme. As for jungle growing that will cost nothing, and it is to be hoped that this will not be a reason for viewing the suggestion with suspicion; it is not everything, that costs much, which is best, and a dog-rose is as lovely as a hybrid orchid.

We do not wish to underestimate the damage done by other species but we, in this conclusion, reiterate our belief that *funestus* (and *aconitus*) in its 'breeding-places,' are the keystone to the arch of the problem. That is why we urge that a dead-set be first made on the perennial streams. We really need in Assam a *funestus* year, just as in England there is a rat-day and a primrose-day.

We beg that the crabsticks and mugwumps will no longer have any vogue; they have stopped anything being done for 30 years since something might have been done. We should no longer permit this sort of letter which was written by one of them to us: 'You can imagine what a lot of work malaria stunts would give me, I fear with little lasting good............' and we no longer wish the newly-imported medical officer to have occasion to write 'Preventive medicine seems unknown in these parts' which was written by another friend.

There is no doubt that on estates, if it be not true elsewhere, 'Prevention is better than Cure.' The medical officer's services in the present state of affairs are only of the value they should be, to the European staff, and perhaps to a few progressively-minded Babus. His help to the cooly is but a travesty of medical treatment as we know it; the cooly only wants the Ayurveda, and probably gets it.

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* At the School of Tropical Medicine it is truly astonishing that although malaria is the disease which kills more persons *per annum* in India than any other, yet no provision or endowment was made for its special study as was done for leprosy, kala-azar, diabetes, hook-worm and bowel diseases.

But we can largely impose our Western standards on him through sanitation and along this road lies the best hope for the future.

We have the support for these opinions, which, while possibly generally realised, we have not heard openly expressed, in Dr. D. P. Williams' (*see supra*) lament that the difficulties of 'medical' as apposed to sanitary work on estates are so great, and in his conclusion that there is then all the more need for a comprehensive scheme in combating disease, chiefly malaria.

There is only one other point, and this provoked by that word 'comprehensive'. It is our belief founded on what we have seen in other plantations that local efforts are all that is needed. It is another blessed excuse of the mugwumps that nothing will be of any avail if co-operation be not secured over a large area; while the fact is, the freedom of the British subject need not be taken from anyone by demanding that he combine with his neighbours to carry out a scheme.

Each man now for himself and the devil take the hindmost

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DESCRIPTION OF A *HERPETOMONAS* PARASITIC IN THE ALIMENTARY TRACT OF THE COMMON GREEN-BOTTLE FLY, *LUCILIA* SP.

14

BY

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By C. STRICKLAND, M.A., B.C.,

Assistant to the Quick Professor of Biology in the University of Cambridge.

(*From the Quick Laboratory, Cambridge.*)

With Plates VIII and IX and 2 Text-figures.

A *Herpetomonas* has been previously recorded in species of *Lucilia* by Patton in India (1908) and by Roubaud in the Congo (1908), but neither observer has described the parasite.

The following paper is concerned with the description of a *Herpetomonas* found in two species of *Lucilia* which infest butcher's shops in Cambridge. It is set out under the following heads:

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Note on the digestive tract of Lucilia.

The alimentary tract of *Lucilia* is similar to that of *Musca domestica* and allied Diptera. The oesophagus (Fig. 1) leads to the midgut, which commences at a valve-like organ, the proventriculus. Before reaching the proventriculus, however, it branches to form the oesophageal diverticulum, which is at first tubular, but later ends in a large bilobed sac, sometimes, though incorrectly, referred to as the 'crop.' The midgut is dilated slightly, and is succeeded by the small intestine, which terminates at the point of entry of the Malpighian tubules. The hindgut commences at this point, and ends in a dilated portion termed the rectum, which opens to the exterior at the anus.

In this paper the term 'crop' will be retained only for the sake of brevity.

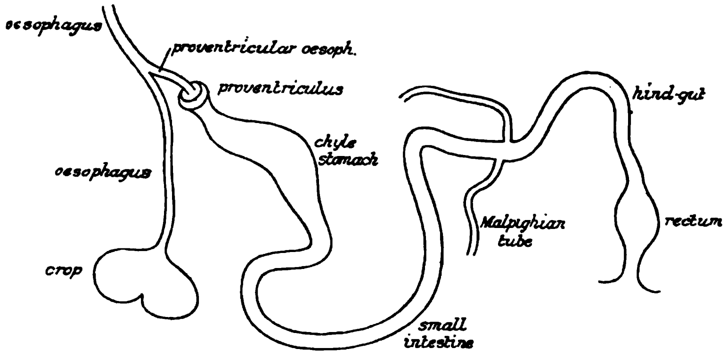

Fig. 1. Showing the anatomy of the alimentary tract of *Lucilia*.

The methods of examination of the gut contents.

The parasites were studied both in the living state and in stained preparations. Living specimens were obtained by dissecting out the flies' intestinal tract in saline solution, removing portions of it to a slide, and protecting with a cover-glass. Stained preparations were made from isolated portions of the gut, opened up in a small drop of salt solution and smeared out in the usual way. They were fixed in absolute alcohol, and finally stained with Giemsa diluted 1 : 20. It was found that a weak solution of the stain gave less diffuse staining than a stronger one.

Morphology.

The general character of the parasite varies in the different regions of the intestine in which it is encountered. The description of the various forms may therefore be conveniently arranged according as they are found (i) in the oesophageal diverticulum or 'crop,' (ii) the midgut,

(iii) the hindgut and rectum, although intermediate forms between the predominant types occurring in each region are occasionally found. The scarcity of such intermediate forms probably depends upon rapid and marked changes of form occurring in the parasites as they pass from one region of the intestine to another.

I. THE PARASITE IN THE OESOPHAGEAL DIVERTICULUM.

In this region, we find (1) *small truncate pyriform bodies* (the so-called cysts), (2) *oval forms without a flagellum*, and (3) *oval forms with a short flagellum*. These forms are connected by intermediate stages, and none of them show any signs of division. They have the following characters:

(1) The cysts, as seen alive, are small truncate pyriform bodies about $3{\cdot}5 \times 1{\cdot}5\,\mu$ in size. Their truncated appearance is due to a small cup-shaped depression in the protoplasm, at what we consider to be the anterior extremity of the cell. The protoplasm can readily be seen to consist of an inner core of endoplasm, invested by a layer of ectoplasm, which however does not cover in the endoplasm at the site of the above-mentioned depression at the anterior end of the cell.

In stained preparations, the ectoplasm, or 'periplast,' appears as a dense blue-staining coarsely sponge-like structure, while the endoplasm is more homogeneous, and appears pale-blue through the periplast, but in cells which have ruptured it can be seen to be stained pale-pink. As was seen in the living cell, the periplast does not completely cover in the endoplasm, but leaves a tiny area where the latter comes to the surface at the truncated end of the cell. The pale-staining endoplasm may be called the *cytopharynx*, and its cup-shaped entrance the *cytostome*. The *lip* of the cytostome consists of periplast. In those cysts which have the most differentiated protoplasm, the lip of the cytostome is inverted, probably by the action of the retracting endoplasm, so that only a minute aperture, or *micropyle*, is left opening into the cytostome. The periplast is thus made to fold over the exposed endoplasm. Embedded in the ectoplasm are the nucleus, blepharoplast, and 'chromidia'; and in the endoplasm traces of a flagellar apparatus can sometimes be seen as a faintly-staining red line. (See Pl. VIII, figs. 1–2, 23–25.)

The *nucleus* is round and placed eccentrically against the side of the cell. It consists of a delicately reticular matrix, which stains pale-pink, and it is devoid of chromatin. It causes a slight projection of the periplast into the cytopharynx.

The *blepharoplast* is a small oval body constantly situated in the periplast at the posterior end of the cytopharynx, and when any traces of a flagellar apparatus are present in the cytopharynx, they are seen to run up towards it. In structure, the blepharoplast consists of a homogeneous-looking matrix, which stains rosy-red, and is devoid of chromatin. It can be distinguished from the chromidia, by which it is often surrounded, only by its staining reaction, the chromidia appearing deep carmine.

The *chromidia* consist of a group of deeply-staining carmine-coloured granules, each of which is often as large as the blepharoplast. The group has a characteristic position at the extreme posterior end of the cell, but very often an isolated granule occurs close to the blepharoplast, the significance of which is not obvious. The chromidia are often obscured from view when the periplast is densely stained. (Pl. VIII, figs. 23–25.)

(2) The *oval forms* are connected with the cysts by intermediate forms which are about the same length as the cysts, but broader. The typical oval forms are longer and possess a pointed anterior extremity, corresponding in position to the cytostome of the cyst. In stained specimens the protoplasm is seen now to be undifferentiated into the periplast and cytopharynx. The chromidia are scattered throughout the cell, and the nucleus and blepharoplast are partly chromatinised. It is consequently very difficult to distinguish the blepharoplast from one of the chromidia, but it lies to the side, or in front, of the centrally placed nucleus. (Pl. VIII, figs. 1–2.)

(3) *The oval forms with a flagellum* differ from the preceding forms only in possessing a free flagellum, the length of which equals that of the body of the cell, and in the nucleus and blepharoplast being more chromatinised.

The flagellar apparatus consists of (i) a rigid rod-shaped and heavily chromatinised body, the rhizoplast, lying between the blepharoplast and the anterior end of the cell, but having no chromatin connection with the blepharoplast; and (ii) the young flagellum, which consists of a delicate prolongation of the cell-protoplasm, staining pale-pink, investing a filament of chromatin, staining deep carmine, which runs into the anterior end of the rhizoplast (Pl. IX, fig. 6). In those cells possessing a divided rhizoplast, two chromatin filaments may be present in the protoplasmic prolongation of the cell, each arising from one of the halves of the rhizoplast (Pl. VIII, fig. 5). Usually the blepharoplast shows no signs of division, but forms may occur in which it has divided, although there are no signs of division elsewhere.

Other forms may be seen with two chromatin filaments in the flagellum, as described above, but with no signs of division in the rhizoplast. (Pl. VIII, figs. 3–6.)

II. THE PARASITE IN THE MIDGUT AND SMALL INTESTINE.

The parasites found in this region of the gut are quite distinct from those seen in the 'crop,' and intermediate forms are difficult to find.

Both in living and stained specimens, they are seen to be in a state of very active division, so that a portion of the gut sometimes contains literally myriads of them.

In the living state, the form of the parasite is seen to be very variable, but often it is like a greatly elongate cone with the flagellum at the base of the cone. The flagellum is long and wavy. The body of the cell measures 15 to $20\,\mu \times 3\,\mu$; the flagellum is 25 to $40\,\mu$ long. Dividing cells are broader.

The following structural details are observable in stained specimens:

(*a*) The cell protoplasm is finely alveolar and stains bright-blue, there being no differentiation into periplast and endoplasm. It contains the nucleus, blepharoplast, rhizoplast, and chromidia; and forms a fine membrane about the chromatin of the flagellum. In dividing cells the protoplasm has a coarser structure.

(*b*) The nucleus is spherical and situated in the centre of the cell. It consists of a reticulate achromatinic matrix, staining pale-pink, in which lies a network of chromatin, thickened at the nodes of the reticulum. The following stages in the structure of the dividing nucleus may be observed:

(i) The nucleus consists of the achromatinic matrix without the finer parts of the chromatin network, so that the latter appears coarser and the nodal points thicker. (Fig. 2 *b*.)

(ii) The nucleus has an oval form, and lies across the cell; the chromatin granules are aggregated around the periphery of the nucleus, and also in a transverse line across its longest diameter. (Fig. 2 *d*.)

(iii) The nucleus shows chromatin granules which have become flattened out, forming a peripheral ring of chromatin, and a transverse band lying nearly across the nucleus, but not touching the peripheral ring. (Fig. 2 *f*.

(iv) In this stage the transverse band of chromatin is divided into two equal parts. (Fig. 2 *g*.)

(v) The nucleus is seen to have divided into two spherical daughter nuclei, each with a peripheral ring of chromatin and a short transverse band of chromatin. (Fig. 2 *h*.)

(vi) The further stages are represented by nuclei in which the chromatin network is reformed by fragmentation of the chromatin bands and the dissemination of the fragments to form a fine network in the nucleus. (Fig. 2 *i*, *k*, *l*.)

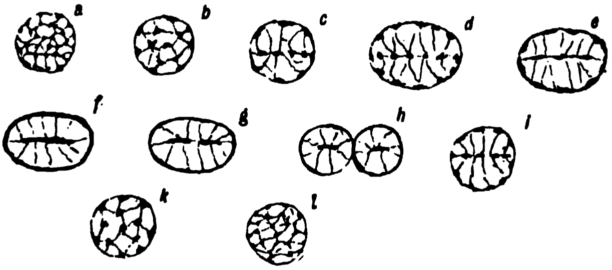

Fig. 2. Showing the structure of the dividing nucleus of *Herpetomonas luciliae*.

(*c*) The blepharoplast lies midway between the nucleus and the anterior end of the cell, and is of very variable form (Pl. IX, figs. 1–14). In the 'resting' cell it is apparently a homogeneous mass of chromatin; but in dividing cells, the chromatin splits into two halves, which separate and reveal a lighter-staining intervening matrix.

(*d*) The rhizoplast is in the same position as in the 'crop'-forms, but it is thicker and contains more chromatin, and therefore stains very densely. In some forms the rhizoplast is split longitudinally, and in such cases it may be connected with either one or two chromatin filaments in the flagellum.

(*e*) The flagellum, in 'resting' cells, consists of a chromatin filament surrounded by an extension of the cytoplasm, as in the 'crop'-forms. In some cases, whether the rhizoplast be divided or not, the flagellum contains a second filament of chromatin which has no connection with the first filament. The two filaments, which may be very different in length, are connected together by a pink staining cytoplasmic membrane. In some of the forms containing two chromatin filaments the membrane stretched between the filaments is split either proximally, or distally, or both; and in other cells the division is complete, the cell then possessing two independent flagella. In such cells none of the organella may be divided, a unicellular biflagellate creature being the result, but these forms are very rare. In yet other cells we find a cell, incompletely divided, with *four* chromatin filaments in the flagellum, which must be interpreted as cells in which redivision is taking place prior to the separation of the daughter cells.

(*f*) The chromidia are scattered throughout the cell. Relatively to the other structures they are very much smaller than in the 'crop'-forms. The more actively dividing parasites seem to contain the most granules.

The structure of the parasites in this region is therefore very variable, in that they do not form a gradual series of forms. This indicates that the sequence of division of the various organella is not uniform; for instance in some cells only the flagellum has divided, in others only the rhizoplast. (Pl. IX, figs. 1–14.)

III. THE PARASITE IN THE HINDGUT AND RECTUM.

In this region the forms of the parasite fall into three main groups, which are connected by intermediate forms: (i) large oval flagellates, (ii) round flagellates, (iii) small oval forms, or 'cysts.' None of these show any signs of multiplication, either in living or stained preparations.

(i) The *large oval* forms are found in the hindgut immediately below the opening of the Malpighian tubes. They are less numerous than the other types, probably because this stage is very transitory. They measure 8 to $10\mu \times 2$ to 3μ. The cytoplasm, as in the midgut forms, is not differentiated into periplast and cytopharynx, and stains pale-blue. The nucleus, blepharoplast, and flagellar apparatus contain less chromatin than the corresponding structures in the midgut forms, and the matrix of the nucleus, which stains pale-pink, is more evident. The blepharoplast still occupies the same relative position to the nucleus as in the midgut forms, that is, well in the front end of the cell. The flagellum is much shorter than in the midgut forms, and stains less deeply owing to its being partly dechromatinised. The chromidia have a tendency to become grouped in the posterior end of the cell. (Pl. IX, fig. 7.)

(ii) The *round* forms are chiefly found in the posterior part of the hindgut, and in the rectum. In this category we include forms which are not round but oval, for the only difference between them is their shape. They are of the same length, about $5\ \mu$, but whereas the round cells are about 5μ in breadth, the oval cells have contracted to about

2μ. The latter are very abundant indeed in the rectum. It appears that the large oval forms become rounded up, and that they then contract from side to side.

The cytoplasm is differentiated into ectoplasm and endoplasm: in forms probably representing a later stage of development, the differentiation is extreme, a very dense-staining periplast being formed. The appearance thus presented is the same as that of the forms occurring in the 'crop,' which have been described above as cysts. The nucleus, blepharoplast, and flagellar apparatus have even less chromatin than in the large oval forms. The nucleus, blepharoplast and intracellular part of the flagellar apparatus may even lose all their chromatin; the nucleus then staining pale-pink, the blepharoplast rosy-red, and the intracellular flagellum being invisible, probably because the dense periplast obscures such a delicate structure.

The blepharoplast is now situated to one side of, or even just behind, the nucleus which is slightly eccentric. The rhizoplast as long as it contains chromatin is seen to follow the blepharoplast as this passes backward, although there is no visible connection between them.

The extracellular part of the flagellum is faintly stained but is seen often when the intracellular part is invisible. The chromidia are very large in those cells in which the organella have lost most chromatin: they are often with difficulty distinguished when the periplast is densely stained. A single chromidium away from the others is sometimes seen close to the blepharoplast. The others are grouped at the posterior end of the cell in the periplast. (Pl. VIII, figs. 10–22.)

(iii) The *cysts* are found in the lower end of the rectum in very great numbers, but are always discrete. They have been described above under the 'crop'-forms. (Pl. VIII, figs. 23–25.)

Taxonomy.

The parasite above described must be classed with those described by Prowazek, Patton, Mackinnon, and others, and named *Herpetomonas.*

It has been recently suggested by Miss Mackinnon (1910) that there has been needless subdivision of this genus into species without consideration of the adaptability of perhaps one species to various hosts. I had this in mind at the time (1909) of conducting the observations on this parasite, and accordingly dissected some *Musca domestica,* and *Calliphora coerulea,* from the same source, two butcher's shops, as the *Lucilia,* but was unable to find herpetomonads in them.

This species is therefore probably distinct from *H. muscae domesticae* at least, and I therefore propose for it the name *Herpetomonas luciliae.*

It has been said that such parasites as these are 'biflagellate,' because certain forms appear to have two flagella. The appearance, however, is deceptive, and these parasites in reality only have one flagellum in which the two chromatin filaments stain very prominently. The term 'biflagellate' is only applicable to those forms, rare in *H. luciliae,* in which there are two *free* flagella.

Prowazek, Chatton, and others would use this inconstant form of the parasite—the so-called biflagellate form—with which to define the genus *Herpetomonas,* and would separate it from *Leptomonas,* which has a slightly different type of flagellar apparatus.

Miss Mackinnon, however, has shown that a 'Leptomonas' in the larval host may become a 'Herpetomonas' in the imaginal host, and that the difference in the flagellar apparatus is only a matter of the time at which the flagellum divides. It seems therefore wrong to class these parasites into two genera.

They would thus all be named *Herpetomonas,* sensû Pattoni, who defines them as having no undulating membrane and the blepharoplast near the anterior end of the cell.

The method of infection of the fly.

The experiments performed in order to discover how the parasite is transmitted from fly to fly were conducted in the following manner.

An attempt was made to raise 'clean' flies in the laboratory, but owing to the cold summer (1909), and also because the experiment was begun rather late in the year, the pupae obtained did not hatch out. Recourse was therefore had to flies caught wild, and in estimating the results obtained with these it was borne in mind that 76 °/₀ of them had been previously found infected.

The flies were kept in a large fly net, but they were with difficulty kept alive. They were fed on fresh meat contaminated with the rectal parasites of other flies, and afterwards were killed at definite intervals and examined for parasites.

The result was meagre, but three flies which had been fed as described above showed actively growing cysts in the 'crop' two days later; whereas of flies examined straight from nature, I never found one containing these forms in the 'crop.'

The fact that rectal cysts are found in the 'crop' of the fly indicates that the parasite is transmitted from fly to fly by contamination of its pabulum.

Hereditary transmission does not take place. Several larvae from eggs laid by infected flies were dissected, but parasites were never found. Neither do these larvae become infected if they feed on contaminated meat, this being in contrast to Miss Mackinnon's observations (1910) on the life history of *Herpetomonas* from dung-flies.

In the case of *H. luciliae*, therefore, I conclude that infection is usually produced in the imago by its feeding on pabulum that has been contaminated with cysts, derived from other infected flies.

Biology.

Movement. The parasites that occur in the 'crop' and hindgut, each possessing only a short flagellum, have little power of progression. The flagellum waves feebly to-and-fro, but without causing much movement. Those with a long flagellum, as in the midgut, can only with difficulty be kept within the field of vision, the flagellum being generally directed forward under these circumstances. The 'cysts' are quite inert, floating about in the fluid in which they are examined.

Growth and division. At various stages in the life-history of the parasite, the relative rapidity of its growth and its change of structure can be estimated by noting the comparative numbers of the parasite at that stage. In the 'crop' the cysts and the oval forms without a flagellum are the rarest, so it must be assumed that these forms are the most rapid in their growth. In the midgut the parasite does not remain long without division; while in the hindgut and rectum the cysts and the small oval forms with a flagellum are found in enormous numbers, and consequently represent the slowest stage of growth.

It seems therefore that rapidity of growth and change of structure is most rapid in the upper part of each subdivision of the gut, while at the lower end it is slowest and there is not much change of structure in the parasite. Division only occurs while the parasite is in the midgut.

Individual variability. The study of stained specimens of the parasite shows that it is very variable in structure, particularly in the forms in the midgut, which we may regard as at the most plastic stage.

Physiology.

Cytoplasm. The cytoplasm of the midgut forms shows no differentiation in structure, and therefore its function is probably generalised; but in the forms occurring in the 'crop' and hindgut, it is more or less differentiated into ecto- and endoplasm. The ectoplasm forms the periplast, the function of which is doubtless to resist desiccation of the cell. It is accordingly most marked in the cysts which, when voided in the excreta, have to withstand the effects of atmospheric drying, perhaps for long periods. From its structure it seems to be formed out of the more solid spongy elements of the cytoplasm. When water and food are plentiful, as in the 'crop,' the periplast soon disappears, which proves that this structure is ill-adapted for the absorption of water and nutritive substances. On the contrary, in the hindgut, where the excreta of the fly are more and more concentrated, the periplast is rapidly formed.

The reduced metabolism of the cell is then carried on by the endoplasm, acting through the small area which comes to the surface of the cell at the 'cytostome.' The endoplasm is thus well-named the cytopharynx. It seems, from its structure, that this endoplasm is derived from the more fluid 'hyaloplastic' part of the cytoplasm.

Later on, as shown by the movements of the chromidia, the ectoplasm and endoplasm diffuse into one another, and then the whole surface of the cell is available for absorption. The cytostome would be a weak spot in the armour of the cell under conditions of excessive dryness, were it not for the following changes. As more dryness is experienced the cell loses water, thereby causing the cytopharynx to retract, and consequently deepening the cytostomic pit. The effect of this is to invert the periplastic lips of the cytostome over the vulnerable area, with the exception of the tiny micropyle described above. The cell is thus enabled to withstand prolonged desiccation.

Rhizoplast. The function of the rhizoplast seems to be the secretion of the chromatin filament in the flagellum. This is probably effected by the protoplasmic achromatic basis of the rhizoplast. When the rhizoplast loses its chromatin this protoplasmic basis is possibly retracted into the blepharoplast, in view of the close connection which exists between the two structures.

Flagellum. The flagellum, which, it must be reiterated, consists of two distinct parts (i) the protoplasmic basis, and (ii) the chromatin filament, is lost in the following manner. The chromatin filament first gradually disappears, but before this change is complete the rhizoplast has passed backward in the cytopharynx, drawing the flagellum after it. By this means the free part of the flagellum is shortened. It constitutes an *active* shortening of the flagellum. Prowazek has previously described this process in the flagellum of *H. muscae domesticae.* The chromatin filament in the flagellum is then absorbed centripetally, so that in some cells only the chromatin of the intracellular part remains, and, as this process takes place, the protoplasmic basis of the flagellum is retracted *pari passu.* The protoplasmic basis does not remain when the chromatin has been absorbed. This constitutes a *passive* shortening of the flagellum.

These observations on the structure and changes in the flagellum seem to show that the chromatin is devoted to sustaining the flagellum in a filamentous form, which the protoplasm surrounding it has no power *per se* of doing.

The function of movement is, we think, subserved by the protoplasmic basis of the flagellum. It is obvious that the protoplasmic basis is homologous to the undulating membrane of *Crithidia* and *Trypanosoma,* although it has been seen that when the flagellum is drawn up into the cell by the posterior movement of the blepharoplast it does not form an undulating membrane as such but remains within the endoplasm. The undulating membranes of the Trypanosomatidae contain myonemes, so that it is most probable that the function of movement is subserved by the protoplasmic basis of the flagellum. The staining reaction of the protoplasmic basis, or of the *kinetoplasm,* as I propose it be called, is the same as the endoplasm of the cell, and is obviously derived from it, so that it is interesting to see that the more hyaloplastic constituents of the cell are employed for the function of movement.

Chromidia. It has been seen how the size and number of the chromidia vary inversely with the amount of chromatin present in the nucleus and other organella. There can therefore be little doubt that they represent the supply of chromatin for the use of the cell. Swellengrebel has shown that they do not consist of chromatin itself, but of a slightly altered substance named 'volutin.' They probably are concerned in the following manner. When the parasites are undergoing involution the chromatin in the organella is dissolved by the protoplasm and circulates in the cell, and is then taken up by certain plastids, and resecreted as 'volutin,' in the form of the chromidia.

The reverse process takes place when the cell is on the 'up-grade,' the volutin being dissolved and resecreted by the protoplasmic basis of the nucleus, blepharoplast, and flagellar apparatus, respectively.

The process is familiar in certain plant-cells, such as *Spirogyra*, in which the carbohydrate metabolism is similar.

The presence of volutin has been shown by Nuttall (1910), and by Hindle (1910), to be associated also with degeneration of the cell, but in this *Herpetomonas* the most vigorous parasites are seen to contain the most volutin granules, or chromidia.

SUMMARY AND CONCLUSIONS.

1. The parasite described inhabits the alimentary tract of two species of *Lucilia*. In addition it has a resting stage—the 'cyst'—which is passed in the voided excrement of the insect.
2. When the cyst is ingested by a fly it grows in length and becomes flagellated in the 'crop'; in the midgut it greatly elongates and multiplies rapidly by division; in the hindgut it first shortens, then becomes spherical, and finally oval and cyst-like, meanwhile losing its flagellum.
3. The cysts measure $3\,\mu$, the fully flagellate forms $20\,\mu$, in length.
4. The cysts possess a *cytostome* and *cytopharynx*, the functions of which are probably nutritive.
5. The flagellum consists of two parts (i) a cytoplasmic part, which probably subserves the power of movement and which I therefore propose to call the *kinetoplasm*, and (ii) a thread of chromatin secreted by the rhizoplast.
6. The function of the chromatin in the flagellum is probably to sustain a filamentous form.
7. The nucleus divides by a definite process something akin to mitosis.
8. The chromidia serve as reserve material for the supply of chromatin in the cell. They are secreted probably by cell-plastids.
9. I propose the name of this parasite be *Herpetomonas luciliae*, and that the genus *Herpetomonas* should be defined so as to include the forms described as *Leptomonas*.

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EXPLANATION OF PLATES VIII—IX.

PLATE VIII.

Figs. 1–6. Forms found in the 'crop.'

Fig. 1. Oval form without flagellum. The chromidia are scattered throughout the cell. The blepharoplast is at the extreme posterior end. It can hardly be seen in the figure.

Fig. 2. Oval form without flagellum. Nucleus slightly chromatinised.

Figs. 3–6. Oval forms with flagellum.

Fig. 3. Shows the blepharoplast dividing, and a single flagellum.

Fig. 4. Shows the cell with the rhizoplast undivided and the flagellum with two chromatin filaments, between which is stretched the kinetoplasmic membrane.

Fig. 5. Shows the rhizoplast divided, as well as the 'double' flagellum.

Fig. 6. A densely staining form in which the nucleus cannot be seen.

Figs. 7–22. Forms found in the hindgut and rectum. In all the cytopharynx is well-marked, but the nuclei are badly marked in the figures.

Fig. 7. Large oval form with small chromidia.

Fig. 8. Shows the chromidia in a group at the posterior end of the cell.

Fig. 9. Shows the nucleus, blepharoplast and flagellum all more or less dechromatinised.

Figs. 10–14. Round forms.

Fig. 10. Shows commencement of the formation of the cytopharynx, the blepharoplast lateral to the nucleus.

Fig. 11. Shows well the blepharoplast at the posterior end of the cytopharynx. The nucleus shows one of the stages of mitosis.

Fig. 12. Partial retraction of the cytopharynx has occurred.

Fig. 13. The chromidia are grouped at the posterior end of the cell. The rhizoplast is still chromatinised.

Fig. 14. Shows the differentiation of the protoplasm into periplast and cytopharynx.

Figs. 15–22. Oval forms with a flagellum. They all show the further differentiation of the protoplasm, and the consequent formation of the truncate end of the cell. The flagellum is short in all cases and is nearly dechromatinised; it cannot be seen when

it becomes intracellular. The blepharoplast is always at the bottom of the cytopharynx and stains less deeply than the chromidia.

Figs. 23-25. Cysts found in the rectum or 'crop.' The cytostome and micropyle are well seen; also the characteristic position of the nucleus, blepharoplast, and chromidia.

PLATE IX.

Figs. 1-14. Various forms of the flagellate found in the midgut.

Fig. 1. Small cell in a resting condition.

Fig. 2. Cell showing two free flagella, the rhizoplast slightly divided.

Fig. 3. Cell in which the blepharoplast is dividing, showing the achromatic matrix.

Fig. 4. Cell showing formation of a second chromatin filament, with a protoplasmic membrane (kinetoplasm) between the two filaments. Nucleus showing mitotic figure.

Fig. 5. Cell showing division of the blepharoplast and rhizoplast; also the formation of a second chromatin filament.

Fig. 6. Shows division in nucleus, blepharoplast and rhizoplast. Flagellum with one chromatin filament in a thick sheath of kinetoplasm.

Fig. 7. Cell in which the nucleus shows mitosis; the rhizoplast is divided and the flagellum contains two chromatin filaments.

Fig. 8. The blepharoplast is dividing and the flagellum contains two chromatin filaments.

Fig. 9. Cell showing complete division except between the two chromatin filaments.

Fig. 10. Shows clearly the kinetoplasmic membrane.

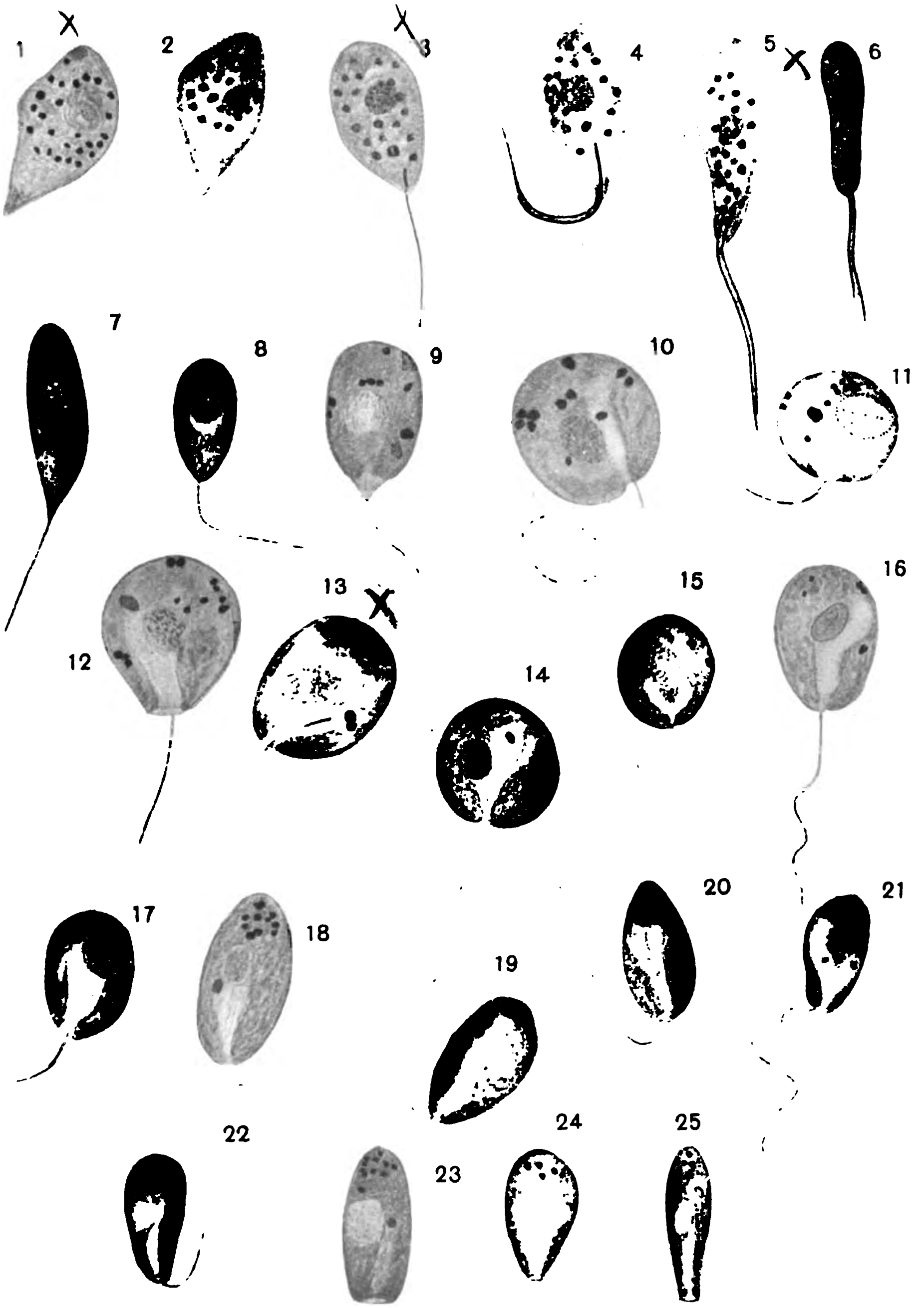
Fig. 11. Flagellum twisted on itself. Rhizoplast and blepharoplast dividing.

Fig. 12. Cell showing complete division of all the organella, but not of the cell protoplasm.

Fig. 13. Complete division of all parts except the flagellum which is only split partially, both proximally and distally, new chromatin filaments being laid down by the side of the old.

Fig. 14. Cell showing a further stage of division. The two cells have completely divided and the formation of the new chromatin filaments is continued. The respective rhizoplasts of the two daughter cells have divided.

PLATE VIII



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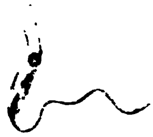

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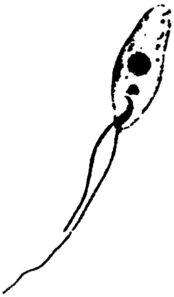

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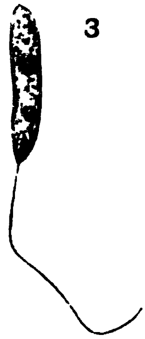

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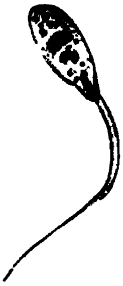

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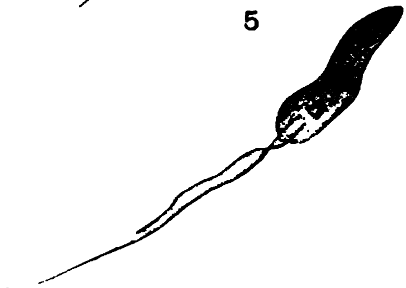

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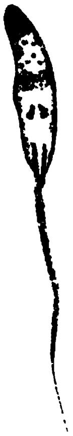

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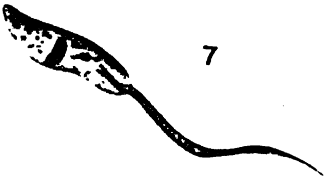

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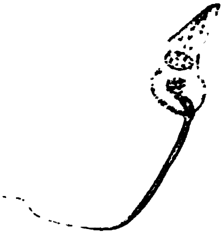

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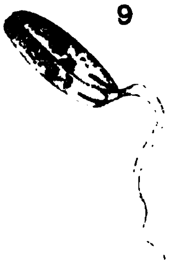

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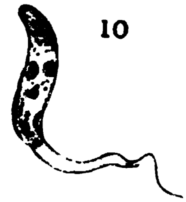

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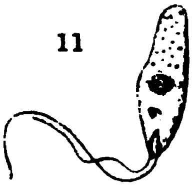

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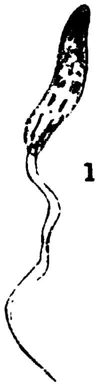

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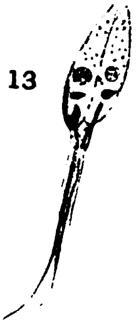

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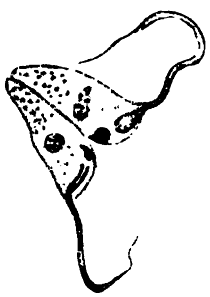

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Strickland and Swellengrebel

1910. Development of Trypanosoma lewisi

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[Extracted from the *Proceedings of the Cambridge Philosophical Society*,
Vol. xv. Pt. vi.]

The development of Trypanosoma lewisi in the Rat Flea (Ceratophyllus fasciatus). By C. Strickland, B.A., and Dr N. H. Swellengrebel. (Communicated by Prof. Nuttall.)

[*Read* 6 June 1910.]

Little is known about the development of trypanosomes outside the vertebrate host, and what is known is liable to Patton's criticism, that asserts that most of the *Crithidia* forms found in different invertebrates and considered to be developmental forms of various trypanosomes are indeed independent forms and have no connection with trypanosomes.

This question being of the highest importance not only theoretically but also from a practical point of view in connection with sleeping sickness, we endeavoured to trace out the life-cycle of *T. lewisi* (provided there were any) in the rat flea (*Ceratophyllus fasciatus*). We chose the rat flea and not the louse (*Haematopinus spinulosus*) because it is clear from the papers of Nuttall, Manteufel, Minchin and one of us (C. S.) that the louse transmits the trypanosomes only mechanically, whereas the flea becomes infective after 7—14 days and remains so for a long time, which fact suggests that a true development takes place.

We infected 83 fleas out of an uninfected lot (bred in the laboratory), allowing them to feed during a period of 12 hours upon an infected rat (10th day of infection). The fleas were then removed from the rat and were fed at intervals of three days by biting non-infected rats through a gauze bag. A control series of experiments was made by feeding 60 fleas (out of the same box as the infected series) on a non-infected rat and by treating them afterwards in the same way. Each day, for 16 days after the first feeding, four to six of the infected and control fleas were dissected and observations were made *in vivo* and in stained preparations.

The control fleas were not always free from flagellated forms. Nothing was ever seen in the living preparations; but by making stained preparations of the different parts of the gut, we were able to detect flagellates in 3·3 $^{\circ}/_{\circ}$ of the fleas. This fact was probably due to an accidental infection, an infected rat having broken into the flea box. But even if we consider the flagellates of the control fleas to be natural forms, we may safely conclude that most of the flagellates found in the fleas fed on infected rats are developmental stages of *T. lewisi*, because the rate of infection was 44 $^{\circ}/_{\circ}$.

Twelve hours after feeding on the infected rat, *T. lewisi* may be found unaltered in the midgut, after 24 hours the blepharoplast leaves its terminal position and approaches the nucleus. The hind-end of the flagellate becomes larger so that the trypanosome appears club-shaped. The nucleus now wanders to the hind-end of the flagellate, and consequently the distance between the blepharoplast and nucleus diminishes gradually, and at a later stage the two pass each other so that the blepharoplast is situated in front of the nucleus, the trypanosome having now a *Crithidia* facies. This change is accomplished in the midgut in 1½ days. The club-shape is now very well marked and the front-end of the flagellate becomes thinner and thinner, the flagellum often staining with difficulty. At last the flagellar end of the parasite atrophies, and the trypanosome is now metamorphosed into an oval-shaped cell, with a short flagellum, the blepharoplast being situated at the side or in front of the nucleus. These "large oval forms," as we may call them, pass into the anterior part of the hindgut, and are to be found there after 3½ days in rosettes. During the following days the large oval forms become rounded, giving rise to the so-called "round forms," which begin to divide actively after 5—6 days. This development is first to be observed in the flea's rectum but afterwards also in the hindgut. We never observed any indication of multiple division, but the parasites arising from the repeated binary division of the round forms hold together with the front ends, so that rosettes are formed. These rosettes are composed of "little oval forms" with short flagella, the blepharoplast being situated just in front of the nucleus. Gradually these become more elongated, the flagellum grows out further, and the blepharoplast passing the nucleus comes to lie in the posterior end of the cell. Since the flagellum follows the blepharoplast, the result is the formation of an undulating membrane. The parasite has now recovered the aspect of a small trypanosome with a particularly large blepharoplast, situated at the extreme posterior end. These forms begin to appear after 7 days but are abundant after 8—10 days and remain so for an indefinite time, probably till the host's death.

All these different types of parasites—little ovals, intermediate *Crithidiae* and small trypanosomes—may divide, so that preparations made during the last stage of infection exhibit a great variety of forms. The small trypanosomes and the *Crithidiae* from which they originate occur in two well-marked types: a slender one and a stout, but united by intermediate forms. We do not know if this is to be considered as a sexual differentiation, but we have never seen any sign of conjugation.

Not being able to find any further stage of development beyond that of "small trypanosomes," either in our series or in the fleas taken haphazard from infected rats, we think it possible that these forms represent the final stages of development. If so, we are unable to say in which way these small trypanosomes return to the rats' blood. We have not been able to find any flagellated form either in the coelom or salivary glands of infected fleas.

At present the broad fact remains established by a proper system of controls that *T. lewisi* undergoes a morphological cycle of development in the gut of its invertebrate host.

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Professor Kofoid
with compliments from
the authors

NOTES ON "TRYPANOSOMA LEWISI" AND ITS RELATION TO CERTAIN "ARTHROPODA."

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BY

C. STRICKLAND, M.A.

AND

N. H. SWELLENGREBEL, PH.D.

FROM PARASITOLOGY, VOL. III. No. 4, DECEMBER 30, 1910

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NOTES ON *TRYPANOSOMA LEWISI* AND ITS RELATION TO CERTAIN *ARTHROPODA*.

By C. STRICKLAND, M.A.
and N. H. SWELLENGREBEL, Ph.D.

(*From the Quick Laboratory, Cambridge.*)

(One Text-figure.)

The following notes on the biology of *Trypanosoma lewisi* are the result of experiments extending over the last two years. They are arranged under the following heads:

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I. THE PREVALENCE OF *T. LEWISI* IN RATS UNDER NATURAL CONDITIONS.

(*a*) *Endemic foci.* Many observers have reported upon the natural prevalence of *T. lewisi* in rats. The statistics relating to this prevalence of the parasite cannot always be compared, because of their being based on observations made in different ways. Thus Lingard (1895) reported on all the rats he examined irrespective of their age, while Petrie and Avari (1909) only noted the presence of the parasite in young rats.

Working in Bombay Vandyke Carter (1887) found 12 °/₀ of the rats infected, Lingard (1895) about 35 °/₀, Petrie and Avari (1909) about 45 °/₀; in the Philippine Islands Musgrave and Clegg (1903) found that about 65 °/₀ of the rats at Manila harboured the parasite at certain times of the year; in the United States Swingle (1907) at Nebraska found four out of seven infected, while Yakimoff (1907) in St Petersburg found a higher proportion infected in the summer months than in the winter but he gives no figures.

Prof. Nuttall kindly allows us to report upon the results of the similar observations which he conducted with one of us (C. S.) during 1909 in Cambridge, where 25 °/₀ of rats (*M. decumanus*) of all ages were found to be infected.

Having regard to the manner in which all of these percentages of infection were obtained it would appear that the prevalence of *T. lewisi* varies considerably in different parts of the world. The figures given by Vandyke Carter, Lingard, and Petrie and Avari for Bombay indicate moreover that the prevalence of the parasite may vary in a given locality from time to time.

(*b*) *Variation of incidence in endemic foci* (*localised endemicity*) Carter, Musgrave and Clegg, and Swingle have observed a marked localised endemicity of the infection, and our observations conducted in Cambridge also reveal the local character of rat trypanosomiasis.

Thus, 20 young rats from Comberton, Cambs, shewed no trypanosomes, while a large percentage from Cherryhinton Brook, Cambs, were found to be infected. We at an early date learnt to know the particular localities from which we could rely for a supply of *T. lewisi* infected rats in and about Cambridge.

Of the Cambridge rats which were infected 12 °/₀ were young, 33 °/₀ adults, and 29 °/₀ old animals.

II. THE RELATION OF ECTOPARASITES TO THE PREVALENCE OF *T. LEWISI* IN RATS UNDER NATURAL CONDITIONS.

Owing to the fairly constant association in nature of *T. lewisi* and fleas, it has been assumed that fleas are concerned to a considerable extent in the transmission of the trypanosome from rat to rat. The association of *T. lewisi* with flea prevalence in rats has been demonstrated very clearly by Swingle (1907) and Nuttall (1908) and more recently by Petrie and Avari (1909) who report upon the examination of a very large material which was rendered accessible to them through the investigations of the Indian Plague Commission. Petrie and Avari found however that the prevalence of *T. lewisi* was inversely proportional to the flea prevalence, although so generally associated with it.

The prevalence of fleas on trypanosome-infected rats from Cherryhinton Brook, Cambs, was very noticeable. On the other hand we failed to find a single flea on rats from Comberton, Cambs, or on 20 rats imported from Berlin, none of which harboured trypanosomes.

The species of fleas which we have found associated with *M. decumanus* infected with *T. lewisi* are *Ceratophyllus fasciatus*, *Ctenophthalmus agyrtes*, and *Pulex irritans* (one on one occasion in company with *C. agyrtes*). We have also captured on dogs *Ctenocephalus canis* harbouring parasites indistinguishable from *T. lewisi*. This species of flea is one which is also occasionally captured on the rat (Tiraboschi).

Some observers believe *T. lewisi* is conveyed from rat to rat under natural conditions by the rat-louse (*Haematopinus spinulosus* Burm.), but no direct association has been recorded.

Of the other blood-sucking ectoparasites which have been found on rats, none are likely to be more than occasional transmitters of trypanosomiasis because they occur rarely on rats as compared with fleas and lice. The ectoparasites of rats are comprised in the following lists:

FLEAS FOUND ON *MUS DECUMANUS*, *M. RATTUS* AND *M. MUSCULUS*.

(Given by Rothschild 1910, p. 89.)

Dermatophilus penetrans, Linnaeus.
„ *caecata*, Enderlein.
Echidnophaga gallinaceus, Westwood.
„ *myrmecobii*, Rothschild.
„ *murina*, Tiraboschi.
„ *liopus*, Rothschild.

Pulex irritans, Linnaeus.
Xenopsylla cheopis, Rothschild.
„ *brasiliensis*, Baker.
Hoplopsyllus anomalus, Baker.
Ctenocephalus canis, Dugès.
„ *felis*, Bouché.
Ceratophyllus fasciatus, Bosc.
„ *londiniensis*, Rothschild (=*italicus*, Tiraboschi).
„ *anisus*, Rothschild.
„ *penicilliger*, Grube.
„ *niger*, Fox.
Pygiopsylla hilli, Rothschild } perhaps not found on *M. rattus* but on
„ *rainbowi*, Rothschild } *M. assimilis* only.
Chiastopsylla rossi, Waterst.
Neopsylla bidentatiformis, Wagner.
Ctenophthalmus agyrtes, Heller.
„ *assimilis*, Taschenberg.
Ctenopsylla musculi, Kolenati.
Hystrichopsylla tripectinata, Tiraboschi.

LICE AND *ACARINES* OCCURRING ON *M. DECUMANUS* AND *M. RATTUS*.

(Given by Tiraboschi 1903—4, p. 161, and by Shipley 1909, p. 68.)

Lice.

Hoplopleura acanthopus, Drury.
Haematopinus spinulosus, Burmeister.
Pediculus capitis, Nitzsch.
Haematopinus precisus, Neumann.

Ticks.

Ixodes ricinus, Linnaeus (larvae reported by Nuttall in England: noted by Neumann).

Hyalomma aegyptium, Linnaeus (larvae from India reported by Nuttall).

Rhipicephalus sanguineus, Latreille (adults identified by Nuttall; see Shipley).

Demodicidae.

Demodex musculi, Oudemans (other varieties have been found on rats (Hahn), and we find one species very common in Cambridge).

Gamasidae.

Laelaps agilis, Koch.
„ *echidninus*, Berlese.
„ *stabularis*, Koch.
Myonyssus decumani, Tiraboschi.
Notoedres alepis, Railliet-Lucat.

III. EXPERIMENTS DEMONSTRATING THE PART PLAYED BY ARTHROPODS IN THE TRANSMISSION OF *T. LEWISI* FROM RAT TO RAT.

We have experimented with fleas (*Ceratophyllus fasciatus* and *Ctenophthalmus agyrtes*), lice (*Haematopinus spinulosus*), bugs (*Acanthia lectularia*), ticks (*Ornithodoros moubata*, nymphs and adults), and a Gamasid mite (*Gamasus* sp.). Only in the case of fleas did any transmission of *T. lewisi* take place by the act of transferring the arthropods from an infected to a non-infected rat. The result of these experiments are recorded in the present paper.

(i) *Experiments with fleas.*

(*a*) *By previous authors.* Rabinowitsch and Kempner (1899) and Nuttall (1908) were the first to regard fleas as transmitting agents of *T. lewisi* and to demonstrate that they convey the infection, and their experiments have been confirmed by Minchin and Thomson (1910).

Minchin and Thomson have recently proved that fleas if fed on an infected rat do not infect susceptible animals until about a fortnight afterwards. This is conveniently described as 'distant transmission' or 'late infectivity of the fleas.' The fleas then continue able to infect for a long time, possibly till the end of their life. From this it is obvious that fleas caught haphazard in nature may have already passed the latent non-infective period and may exhibit the power of distant continuous transmission.

(*b*) *Our experiments.* In all of our experiments the arthropods were allowed to feed on an animal infected with *T. lewisi*, after which they were transferred to a clean animal, a variable interval of time being allowed to elapse before they were transferred from the infected to the clean animal.

We performed 138 experiments with fleas and 22 of these (about 16 °/₀) gave a positive result.

On adding up the numbers of the fleas which were used in the attempts to transmit infection, we find that 324 were used when positive results were obtained, while 1270 were used with negative results. That is, about 80 °/₀ were non-infective. Of the remaining 20 °/₀ concerned with the positive results it is safe to assume that some at least were also non-infective.

We had positive results in 20 experiments and as one flea at least in each experiment must have been infective, at least 20 must have been infective, *i.e.* about 2% of the total number used.

Therefore at least 2% and not more than 20% of our fleas were infective.

We would like here to add the results of flea infection experiments which one of us (C. S.) obtained before the publication of Minchin and Thomson had appeared, since they concerned cases of what may be regarded as 'distant transmission' observed in January, 1909.

Experiment 1. About 20 fleas of two species (*C. fasciatus* and *C. agyrtes*) from an infected wild rat were placed upon a tame white Rat XV. Four days later Rat XV died and about six fleas from it were then placed on a 'clean' Rat XI. Two days later this rat died, and the fleas from it were placed on Rat XVII which became infected in six to ten days. The fleas were therefore infective six days after taking them from the infected rat.

Experiment 2. 17 fleas from an infected wild rat were put on a 'clean' rat for 12 days when nine were recovered and put on a second 'clean' rat. This became infected in eight days. These fleas were therefore infective 12 days after coming from the infected rat. The first 'clean' rat did not become infected.

The result of the following experiment can be interpreted in the way indicated in the second paragraph of this section (distant continuous transmission).

Experiment 3. 15 fleas taken from an infected rat were placed immediately on a 'clean' white Rat XX. In three days four were recovered and put on Rat *a*; similarly after two and one days the fleas were placed in succession on Rats *b* and *c*. All four rats became infected.

The following experiments also prove distant transmission.

Experiment 4. Rat 5 was placed in a box containing fleas which 14 days previously had fed on an infected rat. Rat 5 became infected.

Experiment 5. Rat *d* was used to feed fleas upon 12 days after they had fed on an infected rat. Rat *d* became infected.

Experiment 6. Fleas from an infected rat were placed on a 'clean' Rat *e* seven days after they had fed upon the infected rat. Rat *e* became infected.

Experiment 7. Fleas from an infected rat A were placed on a 'clean' rat B 17 days after they had fed on the infected animal A. Rat B became infected.

Experiment 8. A rat D††† was placed in a flea box from which an infected rat had been taken three weeks previously. Rat D††† became infected in seven days.

Experiment 9. Fleas were fed on an infected animal for an hour and were then fed daily on a series of 'clean' animals from the 4th—33rd day, but none of them became infected. On the 34th day the fleas were put on Rat C which became infected.

It seems therefore characteristic of the flea transmission of *T. lewisi* that it occurs after the lapse of a period during which the fleas are incapable of communicating the parasite. This result is in accord with that obtained by Minchin and Thomson.

We are however inclined to think that infection *occasionally* follows the immediate transference of the fleas to a 'clean' animal, in other words that the so-called mechanical transmission occurs and that it may be followed by the latent or non-infective period which precedes the infective period when distant transmission takes place. In support of this view we would cite the following experiment.

Experiment 10. Fleas were used which had been raised in the laboratory, in the manner advised by the Indian Plague Commission, in large glass boxes in which a flea-infested rat had been placed. Under such conditions the fleas readily multiply in the filth which soon collects in the box. 11 of such fleas were fed for one hour on a rat whose blood had shewed *T. lewisi* for 12 weeks. After 24 and 72 hours the fleas were again fed on a 'clean' Rat D. Rat D became infected in seven days. After this the fleas were fed at intervals on a series of 'clean' animals, but they did not infect the rats again until the 34th day.

In all experiments where we excluded the possibility of 'mechanical transmission' five out of nine rats became infected, but where the possibility was not excluded five out of six experiments had a positive result. The difference in the results observed may have been due to mechanical transmission having been the means of infecting in some of the experiments. The average number of fleas for each experiment was the same in the two series.

If mechanical transmission of *T. lewisi* by fleas takes place we thought that *T. brucei* should also be transmitted mechanically, but the results of six experiments were negative. We allowed fleas to feed on a rat heavily infected with *T. brucei* and then transferred them to clean rats, but in no case did these rats become infected.

We obtained no evidence of hereditary transmission of trypanosomes in fleas although we experimented with a large number (about 60) raised from the larva removed from a flea-breeding box in which an infected rat had been kept for many weeks. The flea imagines produced no infection when placed on 'clean' rats.

Certain conditions may influence the transmission of *T. lewisi* by fleas. For instance Petrie and Avari say that the hot season is the more favourable to transmission because they found fewer fleas *on* the rats and more cases of infection. We think that a favouring factor is connected with the 'wildness' of the flea, for we found that with fleas taken from infected wild rats transmission was obtained much more readily than with those bred and fed in the laboratory. Sir David Bruce and his collaborators (1910) have noted a similar phenomenon in the transmission of *T. gambiense* by *Glossinae.*

Fleas seem to be able to transmit infection whether they have fed on a rat recently infected or on one which has been long infected. We have obtained transmission of *T. lewisi* from a rat which had only been infected one to four days, and from a rat on the other hand which had been infected for three and a half months.

(*c*) *The mechanism of infection.* It has been assumed that fleas infect rats with *T. lewisi* during the act of blood sucking, the parasites being introduced into the host through the fleas' mouth parts. In view of this assumption it seemed somewhat strange that we should only find parasites in the fleas' hindguts: as has been described in a previous paper by us. If we assume that after all the 'small trypanosomes' are the infective agents we could hardly expect them to be carried forward from the hindgut through the pyloric valve and the proventriculus to the mouth parts, for both valves are designed to prevent regurgitation (*see* Jordan and Rothschild, 1908). Moreover we have never found small trypanosomes in the midgut of fleas whose hindgut has been swarming with this form of the parasite. As we have seen we have succeeded repeatedly in infecting rats upon which infective fleas were placed and allowed to feed naturally, that is by being allowed to crawl about and remain upon the host. With the object of learning more about the mechanism of infection attempts were repeatedly made by us to infect rats by allowing infective fleas to bite them through fine gauze, but the results were always negative: thus

Experiments 11—16. A large number of infected fleas (20—30) in six separate experiments were first starved for several days and were then fed respectively on six clean rats through fine gauge. Most of them were observed to feed and could be seen distended with blood afterwards, but none of the 'clean' rats became infected; but when the same fleas were afterwards placed upon another lot of six clean rats and allowed to feed naturally, the latter all became infected.

Experiment 17. Eighteen fleas which had fed on an infected rat E for one hour were fed through fine gauze on a series of clean rats for about a month without any result, but when the only two which survived this time were *put on and left on* a clean rat and allowed to feed naturally, this latter became infected.

Experiment 18. A batch of about 200 fleas were first starved for a few days and were then fed on eight clean rats through fine gauze. After this they were let loose on six other clean rats. Whereas none of the rats on which the fleas fed through gauze became infected, the rats on which they were let loose all became infected.

We are therefore doubtful about the act of biting conveying 'late' infection.

As it seemed to us that late infection was not conveyed by the bite of the flea, we thought that it might ensue from the act of the rat biting and swallowing the flea. We therefore performed the following experiment.

Experiment 19. A 'clean' rat D XII was fed with bread in which was incorporated about 12 fleas which were thought to be infective, as 96 % of their fellows from the same flea-box exhibited small trypanosomes in their hindguts. However no infection resulted.

It is certainly possible that the mechanism of late infection is a *contaminative* one, due to the voiding of the infective agents on to the skin of the rat. It has been stated that during the act of feeding certain fleas squirt excrement from their recta, but we can say that this is not so in the case of *C. fasciatus* or *C. agyrtes* for we have observed many hundreds in the act of feeding and it has never then occurred. Moreover we have never observed that the coat of a rat infested with fleas becomes soiled with their excrement, although we would note here that Brumpt has found the coats of heavily infested mice to be badly soiled by flea excrement. Again, it is very difficult to find any trypanosome forms in the voided excreta of fleas which are even heavily infected with the small trypanosomes in the hindgut and rectum. If infection were due to contamination, one would expect rather a large number of the parasites to be excreted, especially if there were many in the rectum. On the other hand the fact of the presence of the parasites in the hindguts and recta of fleas seems in itself to lend colour to the idea of the infection of the rat being due to contamination. We would also mention here that Manteufel (1909) had no difficulty in infecting rats by placing blood infected with *T. lewisi* on their skins, while Hindle (1910)[1] has lately obtained infection of rats in a similar way by *T. gambiense*. We cannot say what the mechanism of infection really is, but our experiments would appear to indicate that the bite of the flea inoculating the parasite into the rat is not the usual mode of transference.

On the other hand the following experiment seems to indicate that 'mechanical' transmission is effected by the bite.

Experiment 18. Ten fleas from an infected rat were fed within 24 hours on a clean rat *through gauze*, with resultant infection. After this the fleas were similarly fed on a series of animals with negative results for about four weeks. They were then put on another rat and left on it and this became infected.

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[1] In press, to appear shortly in the *Brit. Med. Journ.*

This mechanical transmission by the bite is probably due to the inoculation of the blood forms from the mouth parts of the flea, but there must be even here a close adaptation of the parasites to their environment, for this mechanism is ineffective to transmit *T. brucei* under similar conditions.

Our experiments seem to indicate that the mechanism of infection is not the bite of the flea for 'late' transmission, but that it is so for 'mechanical' transmission.

(*d*) *The relation between the developmental cycle of T. lewisi in the flea and infection by the flea.* It stands to reason that the bodies of potentially infective fleas must contain *T. lewisi* in some form or other, and consequently that the flagellates should be found in the insects upon microscopic examination. It should also be possible to find some forms of the trypanosomes in fleas during the latent period prior to their becoming infective in cases where distant transmission takes place. We have examined a large number of fleas with the object of discovering potentially infective parasites which they might contain, with the following results:

I. Having removed 75 fleas (*C. fasciatus*) from breeding-box 'A' in which an infected rat had been kept for some weeks, they were fed on a series of 'clean' rats during a period of 14 days. None of the clean rats became infected through the agency of the fleas. All of these fleas were dissected and examined during a period lasting for 14 days after they had been removed from the vicinity of the infected rat. In only two of the 75 fleas were flagellates encountered. These two fleas had not been in contact with the infected rat for seven and nine days. The flagellates were small in numbers, occurred in the hindgut, and were of the type described on p. 374.

The examination of the fleas' salivary glands, ovaries, fat-bodies, Malpighian tubes and coelomic fluid gave negative results. The object of first feeding the fleas on 'clean' rats was of course to get some clue as to the presence of infective parasites, or by discovering the mechanism of infection to deduce the whereabouts of the parasite in the insect.

II. Another batch of fleas to the number of 239 were removed from breeding-box 'B' in which an infected rat had been confined for about four weeks. The fleas were divided into two lots (*a*) and (*b*). The fleas in lot (*a*), 20 in number, were dissected and hypothetical infective forms were looked for in all of their organs with the result that 96 °/₀ of the insects were found to contain large numbers of 'small trypanosomes' in the hind gut.

The fleas in lot (*b*), 219 in number, were used for infection experiments on rats. They were placed on 14 clean animals about four days after taking them from the vicinity of the rat infected with *T. lewisi.* About 16 were placed on each rat. Eight of the 14 rats became infected; 45 °/₀ of the fleas distributed on six rats not being infective, *i.e.* although 96 °/₀ of the fleas in lot (*a*) contained 'small trypanosomes' only 55 °/₀ (at the most) of the fleas in lot (*b*) proved infective. We do not know upon what this discrepancy depends.

Since we found no other forms than the small trypanosomes in the hindgut we assumed that these might represent the potentially infective forms, but this assumption gained no support from experiments in which two rats respectively were inoculated subcutaneously and intraperitoneally with 'small trypanosomes' derived from the hindguts of four fleas and yet did not become infected.

We therefore thought that possibly the parasites detected had not reached a stage when they were infective under the conditions in which the experiments were carried out. Although we found no parasites in the infective fleas other than those encountered in the hindgut, an emulsion of the salivary glands and alimentary tracts of 11 fleas from an infected flea-box were respectively inoculated into two rats, but the results were again negative.

We have already said that we think the 'small trypanosomes' in the hindgut of the flea are the final forms which *T. lewisi* takes in the flea, and that it is they which find their way back to the rat when the flea has passed the latent period of non-infectivity and become infective, (late infection).

The forms of the parasite which are found in the flea prior to the 'small trypanosomes' correspond in the time of their occurrence to the time when the fleas are non-infective.

We therefore believe that while the 'small trypanosomes' are inherently able to grow in the rats' blood and infect the rat, the forms which precede them are not capable of doing so. This is we think analogous to the relation of the malarial parasite to its power of infection at different stages of its life cycle, and not to a bacterial culture, which would be infective continuously.

We agree with Novy and MacNeal in the case of *T. lewisi* that the growth in the insect is similar to the growth on agar in 'artificial culture,' and therefore that their conception of the insect being a flying culture tube is to a certain extent justified. The differences which exist are differences of degree, not of kind: the development of *T. lewisi* in the flea proceeds a further stage than it does on agar.

We quote here an observation which may have some significance to the biology of trypanosomes. We found in an experiment that several fleas taken from a heavily infected rat and then fed for a fortnight on a rat which was immune to *T. lewisi* were nevertheless infective; which shews that serum which would have prevented the growth of *T. lewisi* in the rat has no power to do so in the flea.

During the initial period of infectivity of the flea, during the first day when *mechanical* transmission is effected, the trypanosomes are found in the midgut of the flea, and may possibly be the forms which cause infection, perhaps by regurgitation of the gut contents, but we think that probably this mechanical transmission is effected by a 'wet' proboscis, or in some cases by the rat chewing up a flea which is full of *T. lewisi* and becoming infected by way of the mucous membrane of the alimentary tract.

(ii) *Experiments with lice.*

(*a*) *By previous authors.* Many workers have apparently succeeded in transmitting *T. lewisi* by the agency of lice; namely, MacNeal (1904), Swingle (1907), Nuttall (1908), Manteufel (1908), Baldrey (1909), Bensen (mentioned by Baldrey 1909), and Breinl and Hindle (1909).

On the other hand failures to transmit the infection by lice have been common. Thus, MacNeal records but one out of a number of experiments which he performed which was successful. Swingle also succeeded in infecting rats by means of lice, but he does not state how many times he failed to do so. Manteufel (1908) although he seems several times to have been able to transmit *T. lewisi* by lice, yet records 40 failures. Prof. Nuttall (1908) succeeded once (with 60 lice) and failed once (with 40 lice). Baldrey (1909) has succeeded and failed, but gives no figures. Breinl and Hindle (1909) quote three positive experiments but they also had negative results.

Rabinowitsch and Kempner (1899) and Prowazek (1905) could not succeed at all in their efforts at transmission of the parasite.

Further observations are required, but those which we review certainly indicate that transmission experiments by means of lice frequently yield negative results, even when large numbers of lice are transferred from infected to uninfected animals.

(*b*) *Our experiments.* We ourselves have performed 17 experiments all of which gave a negative result, although we modified the conditions of the experiments in various ways.

With regard to these experiments with lice, they have for the most part been conducted with considerable numbers of insects, and we believe that it is only by large numbers that infection is produced. In our 17 negative experiments an average of 67 lice were used in each experiment. It therefore seems more unlikely than ever that lice play any important part in nature in the dissemination of the disease.

It is conceivable that 'distant transmission' may occur in the case of lice in the manner observed in the case of fleas, but there is no trustworthy evidence to prove it. Whilst Baldrey (1909) records one experiment in which he says both mechanical and distant transmission was proved, the experiment has little value since he says that many of his experimental rats were spontaneously infected. Although we repeated Baldrey's experiment, we failed to obtain a positive result. We only carried out one experiment as follows:

Experiment 21. One week after the trypanosomes had disappeared from microscopic observation in the blood of a white rat several lice (about 30) were removed from the rat and after 24 hours, were placed on a 'clean' white rat, but no infection followed, that is, no distant transmission occurred. We will here remark that it was in lice out of this batch that we found the developmental forms of *T. lewisi* described on pp. 378—384 (This *Journal*).

More experiments are needed on this subject. We think that the adoption of Manteufel's or Breinl and Hindle's technique may favour the obtaining of positive results. Manteufel prevented the lice which he used from being eaten by the rat on which they were put by immobilising the rat. Breinl and Hindle transferred the lice which they used to lousy uninfected rats, by which means the transferred lice did not attract the attention of the rat and so get destroyed before they had had time to transmit the parasite to the rat.

(*c*) *The relation between the developmental cycle of T. lewisi in the louse and infection by the louse.* The connection between infection and the morphological character of the ingested trypanosome in lice may ultimately be found to be analogous to that in the flea. The morphological characters of the parasite in the louse have been very variously described by different authors, but a cycle of development terminating with a crithidial or herpetomonad stage seems to have been seen by most observers. We ourselves have recently (1910) described the behaviour of *T. lewisi* in the louse as being exactly similar to that in culture, which agrees with most other workers' observations. We noted that either owing to lice being very short-lived, or to the parasite dying out in lice relatively rapidly, or to some other cause, the growth was found to persist for only a very short time in the louse.

With regard to the coelomic crithidial forms in the louse which have been described by Baldrey, it is possible that they were independent flagellates, and we think that the developmental cycle which the same author describes is partly described from young blood forms of *T. lewisi* ingested by the louse. Baldrey says indeed that the developmental forms are only to be found in lice which have fed on rats just infected with *T. lewisi.*

We would like to emphasize here that the development of the trypanosome in the louse is a very rare occurrence. One of us has previously reported (C. S. 1910) not finding any stage of development whatever in 275 lice, while the other has examined a great number in Holland with negative results. Other observers also have never observed any cycle of development. Thus, Breinl and Hindle say that they examined so many lice from rats infected with *T. lewisi* with negative results that they were about to give up the search when they suddenly came upon developmental forms. Notwithstanding this these authors go on to make the remark that it seemed surprising 'that Nuttall and his co-workers had failed' in the search.

Whatever the cycle of development of *T. lewisi* in the louse may be, it is not very clear as to its connection with infection of rats by lice, as will be seen in the following list of observations made by different authors. Prowazek (1905) and Rodenwaldt (1909) both observed a developmental cycle in lice, but could not succeed in transmitting the parasite by their agency. We have also, as stated above (1910), found a developmental cycle in the louse, but were unable to transmit the parasite with such lice.

Baldrey (1909) however found the developmental forms in lice, and succeeded in transmitting *T. lewisi* by means of them. He correlated what he thought was 'early' and 'late' transmission with different stages in the developmental cycle of the parasite. Similarly Breinl and Hindle (1909) succeeded in transmitting *T. lewisi* from rat to rat by lice and they saw developmental stages of *T. lewisi* in such lice.

On the other hand some of those observers who could find no sign of any development of *T. lewisi* in the louse, were able to transmit infection from infected to non-infected rats by the louse, while others could not. Those who succeeded were MacNeal (1904), Nuttall (1908), Manteufel (1908), and Breinl and Hindle (1909) who at that time could not find the developmental changes. Those who failed were ourselves who at that period could not find the developmental stages, although we have since succeeded in doing so.

(iii) *Experiments with other arthropods.*

We carried out two experiments with *Gamasus* sp., a mite which is occasionally found on rats; two with bugs (*Acanthia lectularia*) and one each with *Ornithodoros moubata* nymphs and adults; but in all cases with negative results. It is of course conceivable that if more of these experiments had been performed a positive result might have been attained.

In all of these experiments the arthropod was taken from a well-infected rat and then placed on a 'clean' rat. The bugs which we used, on being dissected, shewed the developmental forms which we have described on p. 386, while the tick-nymphs were used seven days after they had come from an infected rat and their guts were full of active trypanosomes.

Experiment 22. Four *Gamasus* which were taken from a wild rat infected with *T. lewisi* were immediately placed on a 'clean' white rat, but no infection resulted.

Experiment 23. Two *Gamasus* were taken from a rat heavily infected with *T. lewisi* and put on a 'clean' white rat, but no infection followed.

Experiment 24. About four days after some bugs (*Acanthia lectularia*) had fed on a rat infected with *T. lewisi*, they were allowed to feed on a 'clean' white rat. The result was negative.

Experiment 25. About six days after some bugs had fed on an infected rat, they were fed on a 'clean' white rat, with negative results.

Experiment 26. Several nymphs of *Ornithodoros moubata* were fed on an infected rat and seven days after were fed on a 'clean' animal, but no infection resulted.

Experiment 27. Two adult *O. moubata* which had recently fed on a rat infected with *T. lewisi* were fed on a 'clean' white rat which however did not develop infection.

We thus see that, although we have previously (1910) shewn that *T. lewisi* persists for a definite period in the gut of the bug and the adult tick *O. moubata*, yet we could not obtain infection with these arthropods. It is certainly possible that these arthropods may occasionally transmit the infection, and that this depends on the behaviour of the parasite in their bodies after being ingested, but we have not yet been able to obtain infection with arthropods in which subsequent dissection has shewn the persistence of *T. lewisi.*

IV. DESCRIPTION OF A PECULIAR FORM OF *T. LEWISI.*

We would like to make brief mention here of a curious form of trypanosome found in the rat's blood by one of us. It is characterised by an enormous elongation of the posterior end of the body, the total length of the protozoon being 45 μ. The flagellum is short, and stains deeply red with Giemsa's stain, and though it does not end in the blepharoplast which is very large and spherical, yet no basal granule is evident at its proximal end. The nucleus is situated in the anterior part of the cell and shews the achromatic substance perfectly with disseminated chromatic granules in it. The protoplasm possesses a distinctive band of dense cytoplasm in its middle third, and also a riband of dense protoplasm at the anterior end along the flagellar side. Posterior to the nucleus is a very distinct band of very loose spongioplasm. Disseminated all through the protoplasm are a number of prominent chromidia.

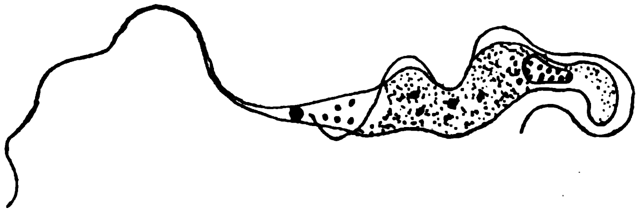

Fig. 1. × 2645.

Wendelstadt and Fellmer have recently found such forms in rats inoculated with *T. lewisi* which have been passed through reptiles and other cold-blooded animals. Its presence in a spontaneously infected rat is therefore suggestive. Thomson (cited by Woodcock) has also previously seen these forms, and other organisms with a considerable prolongation, but not so filiform, have been encountered by one of us (N. H. S.).

SUMMARY AND CONCLUSIONS.

1. *Trypanosoma lewisi* was found in 25 °/₀ of rats caught in and about Cambridge. Of these rats 12 °/₀ were young, 33 °/₀ adult, and 29 °/₀ old. In this region the endemicity was localised to certain areas.

2. Fleas (*C. fasciatus* and *C. agyrtes*) were constantly found associated with wild rats infected with *T. lewisi*. We think that these two species of fleas are the chief carriers of the parasite from rat to rat in this region.

3. We tried transmission experiments with fleas (*C. fasciatus* and *C. agyrtes*), lice (*H. spinulosus*), bugs (*A. lectularia*), ticks (*O. moubata*, nymphs and adults), and *Gamasus* sp., but only obtained a positive result by the agency of fleas.

4. In the transmission experiments the arthropod was transferred from an infected to an uninfected animal. 16 °/₀ of 138 experiments with fleas were successful, 80—98 °/₀ of the fleas used were non-infective, while 2—20 °/₀ were infective.

5. Distant transmission occurs with fleas. This was observed by one of us in January, 1909.

6. 'Mechanical' transmission occurs sometimes with fleas.

7. Infective fleas do not transmit the power of infection to their young.

8. The mechanism of infection in the case of mechanical transmission is by the bite of the flea; in the case of the distant infection it is not so.

9. The infective forms of *T. lewisi* for distant transmission are probably the 'small trypanosomes' which we have described elsewhere (1910). The forms of *T. lewisi* found prior to the small trypanosomes in the developmental cycle are not infective.

10. The development of the trypanosomes in the flea is not interfered with by the flea feeding on a rat immune to the disease.

11. Seventeen experiments with lice were performed, but no transmission was obtained by their agency. 1139 lice were used. No confirmation of Baldrey's infective cycle in lice could be obtained.

12. We could trace no connection between transmission of infection by lice and the presence in them of 'developmental forms.'

13. No transmission of *T. lewisi* by other arthropods was obtained. These negative results had no connection with the presence in the arthropods of 'developmental forms.'

In conclusion we would like to thank Professor Nuttall for his kindness in inoculating our animals and providing us with the material for our experiments, and for his interest and advice in our work.

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17

Further Observations on the Parasites of Simulium Larvae

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FURTHER OBSERVATIONS ON THE PARASITES OF SIMULIUM LARVAE[1]

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SIX PLATES

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INTRODUCTION

The following paper is based upon observations of the parasites of Simulium larvae during the fall of 1911, in the neighborhood of Boston. Although, prior to the spring of 1911, when I found several distinct parasites in these larvae, there were no records of their occurrence in North America, they are, at least in the locality above named, extremely abundant and readily visible to the most casual observer. It would seem, therefore, that these parasites, most of which belong to the order Myxosporidia of the Sporozoa, cannot be generally distributed throughout the United States, since there are no previous records of their occurrence, although Simulium larvae have, in many sections, received very careful attention. The only works which have been published in this country dealing with the Myxosporidia are those of Gurley ('93 and '94), who gave very complete accounts of all the forms then known to occur in fishes. Nothing however has been written in connection with the Myxosporidian parasites of insects. For this reason I have deemed it advisable, before describing in detail the species found as parasites of Simulium larvae, to give a brief review of the order Myxosporidia paying especial attention to the suborder Cryptocystes, or Microsporida. From the observations I have made regarding the Protozoa and their effects upon Simulium larvae I have no reason to doubt that their presence is always fatal to their host, and, since in some cases as many as 80 per cent of the larvae are parasitised, it is probable that the inhabitants of this part of New England owe, to a large extent, their comparative freedom from annoyance by these noxious flies to the abundance of their parasites.

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[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 56.

I wish to express my sincere thanks to Prof. W. M. Wheeler, under whose directions this paper was prepared, both for his help and advice during its preparation, and for a critical examination of the manuscript.

NOTES ON SIMULIUM LARVAE IN THE VICINITY OF BOSTON

The streams in this locality, though usually small, flow rapidly over rocky beds, and are thus eminently suited to the requirements of the early stages of the various species of Simulium. During the spring of 1911 these streams were found to contain immense numbers of the larvae, the most abundant species being S. hirtipes, which occurred in such vast masses that in certain streams hardly a stone could be found which did not support one or more colonies. Large numbers of the larvae of this, and those of an undescribed species were parasitised by Microsporidians. I have published a short account ('11) of the appearance of the infected larvae, and of the external structure of the spores of the species which I found. At the time of discovery all the forms had sporulated, so further details could not be given. By the middle of May all the larvae of the generation under observation had pupated and hatched, and during the summer few larvae were to be seen in any of the streams. Only in a few places could I find an isolated specimen of what I have since found to be the hitherto undescribed larva of Simulium bracteatum Coq.

On October 1 I resumed my search for Simuliid larvae, hoping to find the early stages of the parasites. A number of isolated larvae were observed in most of the streams visited, and it was not long before I found evidence of Microsporidian parasites.

The larvae observed in the streams during the fall of 1911 are as follows:

S. bracteatum; most numerous, occurring in all streams in which any larvae were found, though always living a solitary life. S. vittatum; very few specimens found in one stream. S. hirtipes; not found till the beginning of November after which it was common in some localities.

By the middle of November collecting became extremely difficult as the streams were filled with fallen leaves which had to be removed almost one by one, and examined for larvae, as the latter appeared to prefer them to the stones.

DESCRIPTION OF LARVA AND PUPA OF S. BRACTEATUM COQ.

The adult of this species was described by Coquillett in 1898 from specimens taken at Cambridge, Massachusetts, about seven miles from the locality in which I took larvae and pupae which yielded adults agreeing in all details with his description.

Larva: plate 1, figs. 1 to 7. The mature larva is 6 mm. long and is of a somewhat brownish color. The cephalic fans have about fifty rays which are provided with very short internal cilia, all of which are of the same length. The antennae (fig.

1) are four-jointed, joints 1 to 3 being sub-equal, the fourth joint small and conical. The labium (figs. 6 and 7) has large swollen lateral teeth, and the central tooth is large and prominent while the intermediate teeth are small. On the ventral surface there are usually three strong setae in a row, and two smaller basal setae. The number and position of the setae, however, as in all species examined, are inconstant characters. The mandibular bristles (fig. 3) are not very pronounced. The maxillary palpus is long and slender (fig. 5). The head capsule is never uniformly dark all over; when freshly moulted it is almost white, with a darker median line and dusky latero-basal areas (fig. 2). These latter fuse with one another till the greater portion of the basal two thirds of the capsule is of a deep brown color (plate 5, fig. 1). The thorax and abdomen are greenish gray to brown. The apex of the abdomen is swollen and of a lighter color ventrally. On each side of the swollen portion is a distinct dorsolateral longitudinal furrow. The gills are simple and trilobed.

Pupa: plate 1, figs. 8 and 9. This is 3 mm. long, chestnut brown, turning to black when mature, with four-branched respiratory filaments (fig. 9). The dorsal surface of the posterior margins of the fourth and fifth abdominal segments have eight anterior curved, small, brown hooks. Usually no other segment bears traces of dorsal hooks but the sixth occasionally bears two or three. Ventrally segments 5 to 8 bear at least one pair of obsolete hooks on the posterior margin, and usually there are traces of a second pair of hooks on some of the segments.

Cocoon. This is formed of rather coarse gray silk and does not completely enclose the pupa. It is of the wall-pocket type and is usually found singly, attached to stones or dead leaves.

The pupa of this species is of especial interest, because only two other pupae have been described in which the respiratory filaments are only four branched. One of these is a European and the other a South American species recently described by Lutz ('10).[2]

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[2] Since the above was written Forbes ('12) has described the pupa of a new species, S. johannseni, found in the Illinois River, in which the respiratory filaments are four-branched.

STRUCTURAL PECULIARITIES AND HABITS OF SIMULIUM LARVAE BEARING ON THE SUBJECT OF PARASTITISM

Simulium larvae are particularly difficult insects to study in the living condition because it seems to be impossible to keep them alive for any adequate length of time in captivity. There seem to be two reasons for this. It may be due simply to the fact that the respiratory gills are very small and are unable to extract sufficient oxygen from stagnant water, for it will be remembered that the larvae live only in very fast flowing water. When captive larvae are closely watched they are seen to pass faecal matter very frequently and this activity is accompanied by very evident signs of hunger, for the larvae do not remain stationary as before but turn their heads rapidly in all directions and seem to be searching about on the bottom of the receptacle, in which they are placed, for food. I have even seen them turn and re-ingest their own faeces as soon as they had been expelled. If small Chironomid larvae are present in the water, as is frequently the case, the Simulium larvae will rapidly sieze upon, and attempt to devour them, though owing to the peculiar modification of the mouth parts they never appear to succeed in these attempts. It seems from these facts that hunger is very detrimental to the larva, and the probability of this statement is heightened by the fact that larvae which have died in captivity very rarely have any food in the mesenteron. This region of the alimentary tract is then filled with some secretions, probably digestive, which coagulate in the warm fixing fluid. Although life may be prolonged for several days in healthy larvae it is difficult to keep parasitised individuals alive for more than two days at the most. For this reason I have been unable to carry through any successful experiments on reinfection by the parasites or in transferring them to other species of larvae. Such experiments, will, it seems, have to be performed in the field and could probably be more readily accomplished in the spring when the larvae are most abundant.

It is interesting to note, in passing, that the majority of Simulium pupae fail to hatch in captivity. Many of those which I collected were on leaves which I placed in the water without touching the pupae, so as to prevent any possible damage. Only in three cases, however, in all of which the pupa was almost mature, was development completed, and in all of these the fly failed to reach the surface of the water without wetting its wings.

Mr. A. H. Jennings of the Bureau of Entomology, to whom I mentioned this fact, suggested that it might be due to the inability of the pupa to extract sufficient oxygen from the stagnant water to envelop the contained fly entirely and thus protect it from the water upon its emergence.

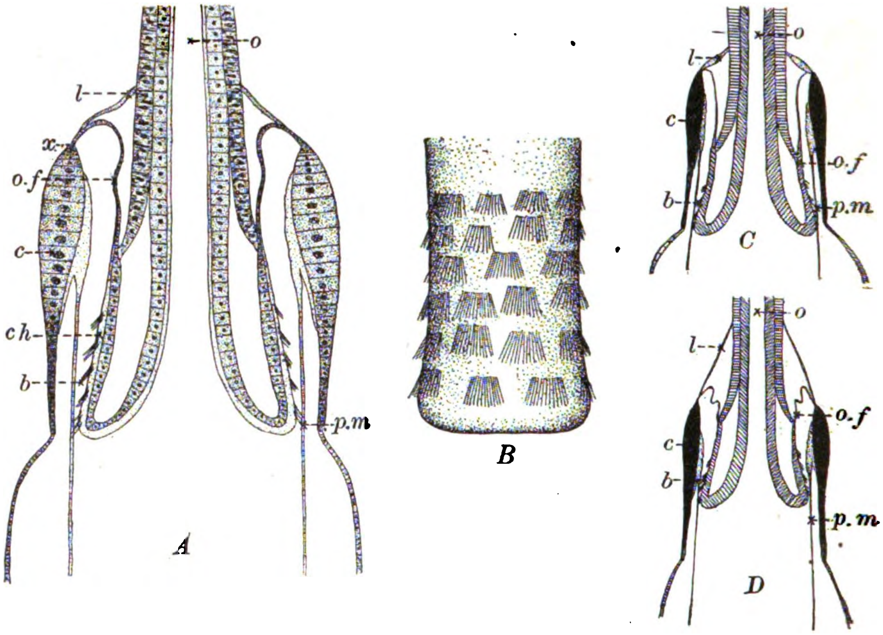
a. The cephalic fans

The cephalic fans of Simulium larvae (pl. 5, fig. 1 a) are extremely specialised organs and are wonderfully suited to enable the larvae to obtain food from the water in a vertical position, thus avoiding the necessity of searching for nutriment in the bed of the stream. In the adult larva of Simulium bracteatum they are composed of about fifty curved rakes, which, when the fans are extended, form two very efficient bowl-shaped strainers, capable of collecting a large quantity of small food-particles from the water as it flows through the small spaces between the cilia of the rakes. In very young larvae, however, these organs are far less completely developed. In a larva measuring only some 0.75 mm. in length they are represented by only about ten widely separated rakes instead of the complete number of about fifty. In two very minute larvae not a single rake was present. Whether this is an abnormal condition or not I am unable to state, but in both cases the alimentary tract was filled with food. I have dissected and sectioned many eggs from different masses, but have been unable to find any in sufficiently advanced stages to show whether the fans are normally formed in the embryo or not. It is a significant fact, however, that neither in the work of Kölliker ('42), nor in that of Metschnikow ('66), which represent the only embryological studies of these insects, are the cephalic fans either figured or mentioned. It would seem, therefore, that the youngest larvae have to obtain their food by picking it up from the stones and débris of the stream, instead of, as at later stages, by simply ingesting what is strained out by their cephalic fans from the rapidly flowing current.

Sections of the alimentary tract of Simulium larvae are of peculiar interest in connection with the peritrophic membrane, which, owing doubtless to the siliceous nature of the food (Diatoms), is exceptionally thick and well developed. As I am led to believe that the presence of this thick membrane is of importance in connection with parasitism, and since it also shows some unique modifications in development, I will describe its formation and structure in some detail.

b. The peritrophic membrane

Text figure 1 shows diagrammatically at *A* a median longitudinal section through the proventriculus and anterior portion of the mesenteron of a larva. From this it will be seen that at the junction of the stomenteron and the mesenteron the former has been pressed into the lumen of the latter to such an extent that its wall has become everted for a considerable distance, so that it folds back upon itself, thus bringing the internal surface of the reflected portion into contact with the first twenty to thirty cells of the mesenteric epithelium. It is from this band of mesenteric cells, which is termed the cardia, that the peritrophic membrane (*p.m.*) is produced. The cells are much modified in size and shape and are sharply marked off from the more posterior mesenteric epithelium. So great is the modification that there has long been a discussion as to whether the cardia is of stomodeal (ectodermal) or mesenteric (entodermal) origin. Miall and Hammond ('00) have shown, from a study of the embryology of Chironomus that in this fly the cells of the cardia are undoubtedly of entodermal origin, and there is no reason to doubt that this is also the case in the allied family Simuliidae. The junction, then, between the stomenteron and the mesenteron is situated immediately anterior to the cardia, and the reflected portion which faces the cardia represents the extremity of the oesophagus. I shall refer to this portion of the oesophagus in future as the oesophageal fold (*o.f.*) of which the end attached to the cardia is the apex and that at the flexure the base. The cells of the cardia are columnar in shape, and in stained preparations have a much greater affinity for such stains as haematoxylin than the remainder of the mesenteron. As is the case with all the mesenteron cells they are glandular in function, and that they are very active is proved both by the quantity of secretion they produce and by their greatly enlarged nuclei.



Text fig. 1 Diagrammatic peritrophogen of a Simuliid larva. *A*, Section of peritrophogen in the normal position; *o*, lumen of oesophagus; *o.f.*, oesophageal fold; i.e., reflected portion of the oesophagus; *x*, junction between the oesophagus and the midgut (stomenteron and mesenteron), *c*, cardia composed of enlarged mesenteric cells which secrete the peritrophic membrane *p.m*; *ch*, thick deposit of chitin on the basal half of the oesophageal fold, bearing stout bristles *b*; *l*, ligament holding cardia in position around the oesophagus. *B*, Surface view of reflected portion of the oesophagus (oesophageal fold) showing imbricated tufts of downwardly directed bristles. *C*, Peritrophogen relaxed. *D*, Peritrophogen extended, showing the bristles, *b*, in contact with the freshly secreted peritrophic membrane.

In a half-grown larva there are three distinct areas in the cardia (pl. 6, fig. 8). The anterior cells are small and closely crowded together. These secrete very little material. Posteriorly there are a number of cells apparently undergoing degeneration since they are much less regular in shape than the intermediate cells. None of these has any secretion attached to the exposed surface. Between these extremes is situated a series of five or six large cells which are very actively secreting a material of a chitinous nature. This, by a process described below, is drawn out as a film into the mesenteron where it functions as the peritrophic membrane.

In young and half-grown larvae all the anterior cells are small and almost functionless, while in mature individuals they are often enlarged and very actively secreting the material of which the peritrophic membrane consists. This, however, is not always the case for in some mature larvae they remain small. They seem, therefore, to constitute a reserve in case more secretion is required, and this reserve is not always drawn upon. That there is a backward movement of the cells of the cardia is indicated by the fact that those cells farthest behind the secreting portion have every appearance of degeneration, and though they are now, as far as can be seen, non-functional, the nucleus is much enlarged, though showing signs of disintegration. These cells also have the appearance of having been pressed out of shape and in some cases degenerate portions of them appear to be sloughing away. Then again there is no peritrophic membrane in the embryo, and it is not improbable that at this stage all the later functioning cells are undeveloped and are crowded together anteriorly. Unfortunately my preparations of the earliest stages obtained do not show details with sufficient distinctness to confirm this supposition.

That the substance of which the peritrophic membrane is composed is chitinous is proved by the fact that it can be boiled in strong caustic alkalies without dissolving. Vignon ('01) showed that the membrane is produced as a fluid which becomes coagulated soon after secretion. From my preparations it seems that the material remains plastic for some time after secretion, and that when in this condition it is very ductile. Plate 6, figure 8 shows that the portion of the cardia which secretes the peritrophic membrane is normally distant from the orifice of the proventriculus, and the question arises as to how the secretion passes backward, as a regular film, to the point where it can first come in contact with the food, to form, when hardened, a continuous membrane which will entirely invest it during its backward passage through the alimentary tract. There is, in every species of Simulium which I have as yet examined, a structure peculiarly fitted to accomplish this, and this structure does not appear to have been seen in any other insect. I failed to locate it in Chironomus but have not examined the larvae of other allied families. In the accounts and figures of the cardia and peritrophic membrane of Culicidae by Thompson ('05) and Imms ('07) this structure is neither mentioned nor illustrated, so it is probably not to be found in the larvae of this family. It will be remembered that, as previously stated, the internal secreting surface of the cardia is faced by the internal surface of the oesophageal fold. The latter, being of ectodermal origin, is lined with chitin, and that lining the basal or posterior half of the fold is very much thickened, so as to be quite rigid (text fig. 1, *A*). It is also beset with strong, backwardly projecting, black bristles, placed in radiating tufts, which are arranged in an imbricated manner over the entire surface of this reinforced area (text fig. 1, *B*). The chitin lining the apical or anterior half of the fold is much reduced and hardly discernable in most larvae. The epithelial cells which secrete this small quantity of chitin are themselves also very small. This renders the basal half of the oesophageal fold, which normally lies opposite the functionless portion of the cardia, very rigid, and the apical half, which faces the secreting cells of the cardia, very flexible. The cardia is held in place around the proventriculus by an elastic ligament (*l.*) formed of connective tissue, which is attached to its anterior extremity, and to the external wall of the stomenteron. This ligament is capable of great extension and enables the cardia to be drawn backwards and forwards over the chitinous surface of the oesophageal fold. These movements were actually observed to take place in a living larva which was examined in a cell slide under the low power of a microscope. Sections also show the different positions the cardia and proventriculus may assume with respect to each other (text figure 1, *C* and *D*). The result of these movements is that the stout bristles on the basal half of the oesophageal fold are brought forward into contact with the newly formed, plastic secretion of the cardia. Owing to the direction in which these bristles are placed they are able to draw a quantity of this material backward when the proventriculus returns to its normal position. This material soon hardens and in a subsequent forward movement of the proventriculus the bristles are withdrawn from the membrane only to become re-entangled with it at a point nearer to its origin, so that any subsequent retrogressive movement will draw more of the secretion backward till the circular membrane thus formed overhangs the orifice of the proventriculus and entirely surrounds the food, which is being passed from here into the lumen of the mesenteron.

I have not examined in detail the musculature which is involved in these cardiac movements, but believe that the whole mesenteron is contracted by a series of external longitudinal muscle fibres which extend along its entire length (pl. 6, fig. 9). This would also account for the wrinkling up of the peritrophic membrane which is often seen in sections of the midgut.

In Simulium larvae the peritrophic membrane is, as previously stated, exceptionally thick and well developed. This is almost certainly due to the fact that these larvae live to a great extent on diatoms and other siliceous matter which, but for the protection afforded by such a membrane, would be very liable to damage the walls of the mesenteron. In these larvae the membrane also remains, for the greater part, intact in the proctenteron, still closely investing the food till it is voided from the anus. That large quantities are continually being formed is evidenced by the fact that the larvae pass faecal matter very frequently and that the faeces are always enveloped in a plentiful supply of the membrane. The membrane is doubtless impervious to anything but liquids as was emphatically stated by van Gehuchten ('90) who concluded that the digestive fluids and the products of digestion pass through it by osmosis. Since this is the case, and since, as before stated, the membrane is exceptionally thick and complete from the proventriculus to the anus, it would seem to be very difficult or even impossible for Microsporidian parasites to gain access to the tissues of the larvae after the membrane has been formed.

c. The relation between these structures and parasitism

In describing the structure of the cardia I drew attention to the fact that there is no peritrophic membrane in the embryo, and it does not seem unreasonable to suppose that in the earliest larval stage the membrane does not entirely surround the food. Indeed I have reasons to believe that the first meal or so of the young larva is the most critical of its life if Microsporidian spores are present in the stream in which it hatches. These reasons are three in number. First, in all cases of parasitised individuals there are indications that the parasite gains admission to the body cavity at a very early period in the life of the larva, for I have never succeeded in finding a full or even half grown larva in which the early stages of the parasite were present. Secondly, owing to the minuteness of the spores, which are comparatively heavy and sink in water, and to the smoothness of their shells, they would readily pass through the fans of somewhat developed larvae which have assumed a vertical position in feeding. In the earliest stages, however, as shown above, it is conjectured that food has to be sought out by the larva which picks up what it can find collected in small depressions in the stones over which it moves. In this way it is extremely likely that it would ingest one or more of the innumerable spores which have been liberated in the water and which have, whenever possible, sunk to the bed of the stream. The third reason is that, as inferred at the conclusion of the last paragraph, some of the first food to escape from the proventriculus may not be entirely separated from contact with the mesenteron by the peritrophic membrane. For if one allow that at this time the membrane is already partially formed, it will not be present far beyond the orifice of the proventriculus and must remain open at the end all the time the food is carrying it backward through the mesenteron. This would give the young germ, liberated from the spore by the action of the digestive juices, ample time to escape from the open end and thus get into actual contact with the epithelium, from which, soon after, it would be entirely cut off during the remainder of the larval life.

CLASSIFICATION OF THE SPOROZOA

The class Sporozoa consists of essentially parasitic Protozoa, from the attacks of which, in all probability, none of the higher forms of life from the annelids up to the vertebrates is immune. The most salient characters of the class are the following:

1. Nutriment is always of a fluid nature and is absorbed by osmosis.
2. Ingesting and digesting organs are never present.
3. Flagella may be present in certain stages of development; these are used for locomotion or attachment but never for nutrition.
4. Certain stages may be amoeboid, but the pseudopodia are used exclusively for locomotion.
5. All forms are capable of sporulation in order to increase the infected area of their host, or by the spores escaping from it to spread the disease.

The Sporozoa are divided by Schaudin into two subclasses as follows:

Subclass I: Telosporidia. Sporozoa in which spore formation ends the individual life; the entire cell then forms spores. Thus the reproductive phase is distinct from and follows the trophic phase. To this subclass belong three orders: Gregarinida, Coccidiida, and Haemosporidia. I shall have occasion to refer to the first of these three orders, namely, the Gregarinida, later.

Subclass II: Neosporidia. Sporozoa in which reproduction begins during the trophic phase and the entire cell is not at once used up in the production of spores. To this subclass belong two orders: Myxosporidia and Sarcosporidia.

The Myxospiridia, with which we are mainly concerned in this paper, have the following characters:

1. The earliest stage of the trophozoite is amoeboid.
2. Spore formation usually begins at an early period and continues during the growth of the trophozoite.
3. The spores are produced endogenously.
4. Each spore possess one or more polar capsules.

There are two suborders of the Myxosporidia, mainly separable on spore characters. These are:

Suborder I Phaenocystes (Gurley) = Myxosporidia *sens. str.*, with large bilaterally symmetrical spores having two or four polar capsules which are plainly visible in the fresh state. Two spores are formed in each pansporoblast.

Suborder II Cryptocystes (Gurley) = Microsporidia (Balbiani), with minute pyriform or oval spores having one polar capsule, which is visible only after treatment with reagents such as weak HNO_3, and in some cases not even then. One (Nosema), or more than two spores are formed in each pansporoblast.

The Protozoan parasites of Simulium larvae all (with the exception of an undetermined Gregarine) fall into the second of these sub-orders, namely the Microsporidia of Balbiani.

TYPICAL LIFE CYCLE OF A MICROSPORIDIAN

The Microsporidian genera are distinguished entirely by their mode of development, and largely by the final stage, i.e., sporulation. A brief account of the development and means of infection of these parasites will be useful before we pass to the generic classification.

Stage I: The germ. This name is applied to the minute amoeboid, motile body which is liberated in the alimentary canal of its host from a spore which has been taken in with food. At the time of liberation the germ has either one or two nuclei. In the latter case the two nuclei soon fuse. A minute vacuole is also sometimes present (Stempell '09). The germ passes between the epithelial cells of the gut and so reaches the blood sinuses. It is now termed a 'planont.'

Stage II: The planont. This body is also minute and neither this nor the preceding stage have been actually found in the great majority of described Microsporidia. The best account is that given by Stempell ('09) of Nosema bombycis Nag. Owing to their intercellular life the planonts are readily distinguished from the following stages which are intracellular. They measure (in N. bombycis) from 0.5 to 1.5μ in length and are bullet-shaped, but have sufficient amoeboid movement to enable them to spread over the body. Under favorable conditions the nuclei can be seen. Division occurs in this stage, often quite actively so that masses of these minute organisms are seen.

Stage III: the meront. The planont enters a cell and at once loses its motility, becoming spherical or oval. In this stage division of the nucleus is rapid. In certain genera each division of the nucleus is accompanied by a division of the protoplasm so that *numerous meronts* are formed. In other genera the nucleus divides independently of the protoplasm so that a multinucleate body is formed, which may attain considerable dimensions. This body is usually termed a 'myxosporidium' or 'trophozoite.' The typical myxosporidium consists of a very clear ectoplasm surrounding a granular entoplasm. The former may be capable of throwing out pseudopodium-like processes which are used only as locomotor organs. Most often the myxosporidium is sessile, and in some genera is capable of encystment. The entoplasm is coarsely granular and sometimes slightly yellowish. It contains numerous rapidly dividing nuclei and in addition may possess fat globules, pigments and one or more vacuoles. In the genus Nosema, where the meronts are numerous and uninucleate, each of them matures directly into a 'spore,' but in all the other genera there are intermediate stages between the meronts and spores. During the meront stage the parasite usually breaks down the cell of the host in which it was formed from the planont, and lives for the remainder of its life as an intercellular parasite.

Stage IV: sporont. A small clearly defined sphere of protoplasm collects around each of the nuclei and its peripheral layer condenses to form a delicate envelope. The subsequent development of the sporont varies in different genera and species. Mercier ('08) interpreted the subsequent developmental stages in Thélohania giardi Henn. as follows: The indefinite nucleus is purified by the rejection of part of the chromatin of which it is constituted. The remaining chromatic matter fuses to form a ring-shaped nucleus which undergoes a division intermediate between mitosis and amitosis. The protoplasm also divides to form two uninucleate bodies. Both of these undergo two subsequent and similar divisions within the membrane of the sporont, so that the latter, which is now termed a 'pansporoblast' contains eight similar bodies or 'sporoblasts,' together with a small quantity of rejected chromatic matter.

Stage V: sporoblast. These eight bodies assume a pyramidoidal shape and their nuclei undergo a somewhat complicated division, at the end of which the sporoblasts contain three nuclei surrounded by a dense cytoplasmic mass. A circular vacuole appears within the cytoplasm and rapidly increases in volume. One of the nuclei is attached to the vacuole. The latter becomes pyriform in shape and the narrowed end comes in contact with the surrounding envelope of the sporoblast. This vacuole is the 'polar capsule' and at the point where it comes in contact with the envelope, a coiled up, evaginable filament is formed within it. In all of the forms I have examined there are two vacuoles, the first of which travels to one end of the somewhat elliptical spore and entirely replaces the cytoplasm of this region to form the vacuole of the spore. The pyriform polar capsule is subsequently formed at the opposite end of the spore, where its location is not very evident on account of its being surrounded by the cytoplasm. It may however increase considerably in size so that it projects far into the vacuole.

Stage VI: spore. At the time when this internal maturation has been accomplished, a very thick, though remarkably transparent shell has been formed around the sporont which is thus converted into a 'spore,' strongly resistent to all external conditions. There is a single minute pore in this shell situated opposite the point of contact with the narrowed end of the polar capsule, and therefore in communication with the base of the filament. The spore thus consists of a very resistant transparent shell containing a single polar capsule, in which is a coiled evaginable thread. Surrounding the base of this capsule is a collar-like mass of protoplasm containing either two or four nuclei, while the apex projects into a large vacuole occupying almost half of the area of the spore. Both Mercier ('08) and Stempell ('09) find that in T. girardi Henn. and N. bombycis Nag. respectively there are two minute nuclei attached to the shell and one similarly minute nucleus attached to the polar capsule.

By means of these spores the disease is disseminated among new hosts. Where the parasite infects the epithelial cells of the gut and other excretory organs the spores escape into the alimentary tract and are passed out of the body with the faeces. If other organs form the seat of attack the spores are liberated, either by forming a tumor through the bursting of which they escape, or they await the death and subsequent decay of their host. Pasteur ('70) showed, in the case of the pébrine of silkworms that the spores can pass into the ovary and thus spread the disease by infecting the eggs. If the host is aquatic, as is most often the case, the spores fall to the bottom of the water, and there remain unchanged till taken into the alimentary tract of a new host, while in terrestrial hosts, as in the silk-worm, the spores are scattered in the faeces of infected larvae and thus contaminate the food on which the healthy larvae are feeding.

As soon as the spores reach the foregut of a new host, the digestive juices set up intrasporal pressure either by causing the shell to contract, or by passing through it by osmosis, which contracts the polar capsule and thus ejects the spirally coiled filament through the pore in the spore shell. Stempell ('09) points out that the filament, owing to the manner in which it is ejected, evidently consists of a hollow tube which is everted when it protrudes from the spore. The filament is often very long, that of the spore of [Nosema] Glugea simulii Lutz and Splendore ('08) and of Glugea fibrata sp. nov., which I have recently found in Simulium larvae, being thirty to forty times the length of its parent spore. The function of this filament has been the subject of much discussion. It was first discovered by Balbiani in 1863, when he attributed to it a function similar to that of the antherozoid of the Cryptogams. In 1882 Bütschli drew attention to the similarity between the polar capsule and its contained filament to the nematocysts of coelenterates. He, however, did not assign to it a similar urticating function, but suggested the now generally accepted theory that the filament serves to attach the spore to new hosts or to their food. It should, however be borne in mind that the filament, so far as known, is not evaginated till the spore has been taken into the gut of a new host, a fact which appears to lessen considerably, if not to entirely nullify the use of this organ, if its function be such as Bütschli surmised. The filament is soon detached from the spore, leaving a small opening in the shell through which the binucleate cytoplasmic contents escape. Stempell ('09) finds that in N. bombycis, where four nuclei are present in the spore, only two of them pass out of the shell with cytoplasm, the other pair remaining behind and degenerating. The small free body thus liberated is the germ, and its nuclei soon fuse to form the single nucleus of the planont.

CLASSIFICATION OF THE MICROSPORIDIA

Stempell '09 has recently revised the classification of the Microsporidia as follows:

Family 1: Nosematidae. The vegetative stage is intracellular and consists of unicellular dividing meronts.

a. Genus Nosema (Nägeli '57). Each meront gives rise to a single spore.

b. Genus Thelohania (Henneguy and Thélohan '92). Each meront gives rise through a sporont to eight spores.

c. Genus Gurleya (Doflein '98). Each meront gives rise through a sporont to four spores.

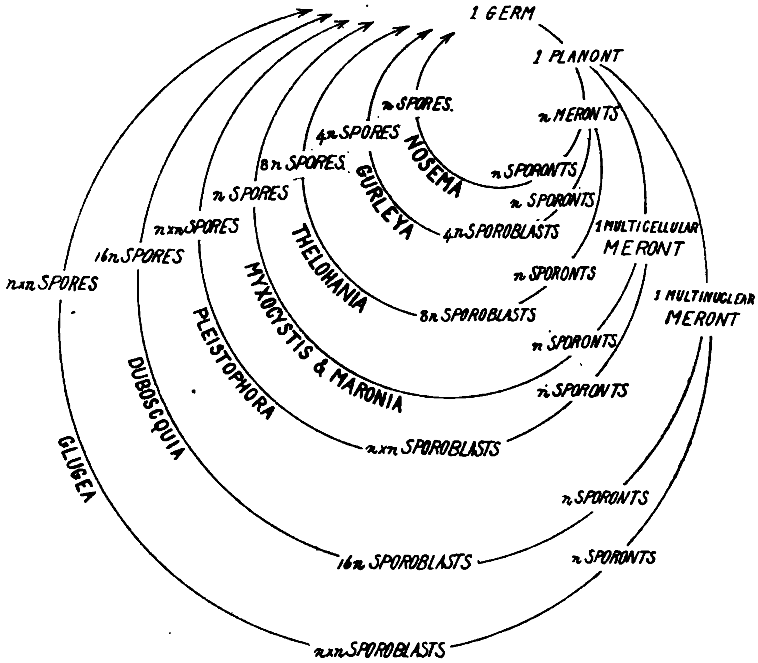
Family 2: Pleistophoridae. The completely mature vegetative stage consists of multicellular, often amoeboid, motile meronts.

a. Genus Pleistophora (Gurley '93). The vegetative stage passes into rounded sporonts from which many spores arise.

b. Genus Maronia (Stempell '09). Spores arise through endogenous budding within the protoplasm of the amoeboid vegetative stage.

c. Genus Myxocystis. (Mrazek '97). Spores arise through endogenous budding within the protoplasm of the vegetative stage whose ectoplasm consists of motionless cilia.

Family 3: Glugeidae. The vegetative stage is multinuclear and motionless, remaining undivided and encysted. Sporonts arise in it by endogenous budding.



Text fig. 2 Diagram showing life cycles of Microsporidian genera

a. Genus Glugea (Thélohan '91). The number of spores formed from a sporont is variable.

b. Genus Duboscquia. (Pérez '08). The number of spores formed from a sporont is sixteen.

For the sake of making clear the life-histories of these various genera of Microsporidia as conceived by Stempell I have constructed the accompanying diagram (fig. 2).

GENERAL HOST RELATIONSHIPS OF THE MICROSPORIDIA

By far the greater number of known cases of Microsporidian infection have been recorded from fish, though infection of crustaceans, particularly of crabs, is not infrequent. In insects about forty cases of infection have been recorded. A great number of the descriptions, however, are very incomplete. All tissues of the host are liable to be the seat of infection, though in fish the muscles are the most favorable, while in insects the fat body usually suffers.

The tissue-infecting forms fall into two categories: (1) *Concentrated,* in which the adjacent tissues form a membrane around the trophozoite, or the trophozoite itself is limited by a definite membrane; (2) *Diffuse,* in which the protoplasm of the parasite and host cells becomes indistinguishably mingled, till the tissue is found to be infiltrated with vast numbers of spores (Nosematidae).

As a general rule, infection by these parasites is not fatal to the host though its vitality is much impaired. When larvae of insects are infected the parasite is often able to pass over into the adult and in the case of pébrine is capable of infecting the ova, so that the disease is transmitted to the next generation.

Insect hosts of Microsporidia

The following list of the insect hosts of Microsporidia is partially adapted from Minchen's work ('03) on Sporozoon hosts, with additions from more recent literature. Doubtful, or incompletely described forms have been in most cases omitted.

Diptera

Anopheles maculipennis, larva	*Thelohania legeri* Hesse ('04)
Chironomus sp., larva	*Nosema chironomi* L. and S. ('08)
Corethra sp., larva	*Nosema corethrae* L. and S. ('08)
Limnophila rhombia, larva	*Thelohania janis* Hesse ('03)
Pachyrhina pratensis, larva	*Glugea stricta* Monz
Simulium ornatum, larva	*Glugea varians* Legér ('97)
Simulium venustum, larva	[*Nosema*] *Glugea simulii* L. and S. ('08)
Simulium ochranum, larva	[*Nosema*] *Glugea simulii* L. and S. ('08)
Simulium bracteatum, larva *Simulium hirtipes,* larva	*Glugea bracteata* sp. nov. *Glugea fibrata* sp. nov.
Simulium vittatum, larva	*Glugea multispora* sp. nov.
Stegomyia fasciata, larva and adult	*Nosema* sp. Simond ('03)
Stegomyia fasciata, adult	*Nosema stegomyiae* L. and S. ('08)
Tanypus varius, larva	*Thelohana pinguis* Hesse ('03)

LEPIDOPTERA	
Bombyx mori, all instars	*Nosema bombycis* Näg.
Catopsila eubule, larva	*Glugea eubulis* L. and S.
Danais erippe, larva	*Glugea erippe* L. and S.
Danais gelippus, larva	*Glugea erippe* L. and S.
Diono juno larva	*Glugea janoris* Los.
Ephialtes angulosa, adult	*Nosema ephialtis* L. and S. ('08)
Gastrophilus neustria, larva	*Nosema bombycis* Nag.
Lophocampa flavostica, larva	*Glugea lophocampae* L. and S.
Mechanitis lysimnia, larva	*Glugea lysimniae* L. and S.
Scea auriflamma, adult	*Nosema auriflammae* L. and S. ('08)
COLEOPTERA	
Balaninus amaryllis, larva	*Glugea stempelli* Pérez ('05)
Otiorhynchus fuscipes, larva	*Mycetosporidium talpi* Legér and Hesse ('05)
ORTHOPTERA	
Periplaneta americana,	*Glugea periplanetae* L. and S.
Termes lucifugus	*Duboscquia legeri* Pérez ('08)
NEUROPTERA	
Ephemerella ignita	*Gurleya legeri* Hesse ('03)

MATERIAL AND METHODS OF STUDY

Simulium larvae from infested streams were frequently collected and brought to the laboratory, but since parasitised larvae could never be kept alive for much longer than over night, the majority were killed at once, a few being kept for dissection.

Killing for sectioning. The best fluid for this purpose proved to be one recommended by W. Kahle ('08) which is made up as follows: water, 30 parts; 96 per cent alcohol, 15 parts; 40 per cent formalin, 6 parts; glacial acetic acid, 1 part.

A fluid recommended by Stempell ('09) consisting of corrosive sublimate (saturated aqueous solution) 2 parts; 95 per cent alcohol, 1 part; and a trace of acetic acid, also gave good results, but for subsequent staining with iron haematoxylin was inferior to Kahle's fluid. Either fluid was used by being brought to a temperature of about 70° C., when the larva was immersed and allowed to remain in the cooling fluid for about fifteen minutes. To insure penetration the larval skin was punctured with a fine needle as soon after death as possible.

Killing for smear preparations. Kahle's fluid poured over the smear gave good results, but a method recommended by Perrin

'06 for Pleistophora periplanetae proved somewhat superior before staining with an eosin-azure mixture. With this method a smear is made on a cover glass and allowed to dry. It is then immersed in absolute alcohol for ten minutes and again dried. Perrin then stained over night in one of Giemsa's preparations, after which the slide was washed, dipped for a moment in absolute alcohol, rewashed and mounted in cedar oil.

Stains. For general histological purposes Heidenhain's iron haematoxylin in combination with orange-G proved to be the most satisfactory. This combination, however, is almost useless for studying the nuclear matter of the spores, for the cytoplasm takes up the haematoxylin as readily as does the nuclear matter and it cannot be again washed out. The usual stain for this purpose is Giemsa's 'Eosinazur' stain. The blue of this mixture however proved to be too intense. Other Giemsa preparations were not altogether satisfactory and I finally found it preferable to stain separately with azure II and eosin A.G. and to differentiate by overstaining with a 0.8 per cent aqueous solution of azure II, rapidly washing with water and then dehydrating. The absolute alcohol contains a saturated solution of eosin and the slide can be left in it for about five minutes after which it is immersed in xylol and examined in it as a medium. The nuclei of the spores have a strong affinity for eosin which can be readily washed out of the other tissues with water. If the blue be too intense it can be washed out in alcohol. By overstaining with both azure and eosin and bearing in mind that the former can be washed out most readily with alcohol and the latter with water, one can, with a little practice, get both stains nearly counterbalanced.

THE MICROSPORIDIAN AND OTHER PARASITES OF SIMULIUM LARVAE

The earliest account of a Microsporidian parasite of Simulium larvae that I have been able to trace was that of Léger '97. He describes a Glugeid, G.varians, parasitising the larvae of S. ornatum, a species common in France. In 1904 Lutz and Splendore described various forms of Glugeids found in the larvae of S. venustum and S. ochraceum. In a further discussion of these forms in 1908, they regarded them all as varieties of one species, which they designate as Nosema simulii. The locality in which this presumably South American species was taken is not stated. In a subsequent paper by Lutz ('09) on the Simulidae of Brazil, he mentions the presence of Nosema sp. in the larvae but gives no details. The only other account seems to be the the one which I published in 1911. In that I described the external appearance of the spores of several undetermined species which infest the larvae of S. hirtipes and an undescribed Simulium larva common in the streams in the vicinity of Boston, Massachusetts.

During the fall of 1911 I found three further well-marked forms in the Simulium larvae of the same locality. One of these resembles the form described by Léger. His description, however, is very short and is not accompanied by figures. The filament of the American species is about twice as long as that of G. varians. Another form resembles somewhat Lutz and Splendore's figures of [Nosema] Gluguea simulii but here again the filament of the latter species is about six times the relative length of the American species. Probably, with a fuller account of the development of these exotic species, other differences would be found, especially in the case of G. varians, for it is unlikely that the same species of Microsporidian should occur in both hemispheres parasitising larvae of such specialized and confined habitats as those of Simulium. It is true that this genus of Diptera is cosmopolitan and is recorded by Grünberg ('07) as occurring even in Lapland and Greenland, but since none of the European species has been recorded from America it is very unlikely that their parasites, which also occur in both hemispheres, should be but varieties of the same species.

The Microsporidian species under consideration apparently all fall into the genus Glugea as defined by Thélohan ('91). In 1892 Henneguy erected the genus Thelohania of which I have been unable to obtain the original description. The sporulation of the species I have described as Glugea bracteata is typical of the genus Thelohania according to Gurley's ('94) interpretation, which, from the same writer's modified definition of Glugea, debars it from this genus. The early stages are however typically Glugeid so that I have provisionally placed the species in this genus. I shall discuss this more fully after describing the species.

During February, 1912, I found a species of Glugea infesting the larvae of Simulium sp. in streams around Port of Spain, Trinidad, B.W.I. The spore formation in this species was polysporic in which respect it resembled S. multispora, of which a description is given further on in this paper. This species was not abundant, although I found some half-dozen infected larvae.

Other parasites which I have found infesting Simulium larvae in the neighborhood of Boston are a nemathelminth belonging to, or near, the genus Mermis, which, during the spring of 1911, parasitised some 25 per cent of the larvae of the streams in which it occurred. As pointed out in my former paper ('11) on this parasite, its presence is fatal to the host in every case. I have since found that the presence of these worms in Simulium larvae was also mentioned by Lutz ('09) in his work on Brazilian species of the genus.

During the fall of 1911 another parasite was found infesting the majority of larvae in certain streams and, as I shall show, its presence is evidently fatal to the host. I sent preparations of this parasite to Professor Calkins, and he informs me that it is probably a species of Gregarine. I have not studied it in detail and shall confine my remarks in the sequel to its general appearance and its effect on its host.

GLUGEA BRACTEATA SP. NOV.: (Plate 2)

Macroscopic appearance. In later stages this parasite is present in the body cavity of its host as a large irregular white mass, sometime measuring as much as 2 mm. in length. This mass may consist of one very large, or several smaller myxosporidia which together only occupy as much space as does the single large myxosporidium. It is usually confined to the posterior portion of the abdomen, which becomes much distended, owing to the large mass of the parasites which is most voluminous latero-ventrally to the alimentary canal (pl. 2, fig. 1).

Microscopic structure. If a portion of one of these milk white masses be dissected and placed in a drop of water under a cover glass it will be seen with the high power of a microscope to consist of many different minute bodies, the most abundant in late stages being small aggregates of eight short-oval spores (pl. 2, fig. 11). If the cover glass be gently pressed these aggregates will open up and take on an appearance like that shown in plate 2, figure 12. By gently rolling these aggregates between the cover glass and the slide they can be broken up and the now separated spores are seen to be nearly uniform in size, short elliptical in shape and to measure about 3μ x 2.5 – 2.7μ. If weak iodine solution be added to the water in which they are floating a few of them will eject a short filament measuring about six times the length of the spore (pl. 2, fig. 18).

Life history. In order to trace the developmental stages of this parasite, sections not over 5μ thick must be prepared and stained, preferably with iron haematoxylin and orange-G. The earliest stages, viz., the planont and early meront, are still unknown, but I shall attempt to trace their conjectural development in a later paragraph.

Myxosporidium. At the time of discovery of the parasite the mass of meronts, or, as this is now better termed, the myxosporidium, consisted of a multinucleate mass of protoplasm measuring some 2 to 3 mm. in length, in which no definite ectoplasm and endoplasm could be distinguished. The central, and larger, portion of the myxosporidium had already sporulated and contained ripe spores; surrounding this area were all stages of development up to the as yet almost undifferentiated thin layer of protoplasm which still persisted around the edges of certain parts of the mass and represented all that was left of the true meront stage. In the stained section there is a very definite differentiation between the ripe spores and the early stages, for the former stain very intensely with haematoxylin, whereas the latter are left practically unstained by this, though showing a marked affinity for the orange-G, so that the section takes on the characteristic appearance shown in plate 2, figure 2.

Sporonts. These are typically formed by the condensation of the myxoplasm around the numerous nuclei of the myxosporidium and this results in the formation of small globular bodies, around which the protoplasm hardens to form a fine constricting membrane (pl. 2, figs. 3 and 5). At times, however, they appear to be budded off from the peripheral myxoplasm toward the center of the mass, as shown in plate 2, figure 4. In either case the resulting sporont is the same, consisting of a spherical body mesuring about 5.8μ in diameter. In this and in all subsequent stages up to that of the spore it is extremely difficult to stain the nucleus, for the various nuclear stains tried washed out of the chromatic material almost if not quite as readily as out of the protoplasm. Development, however, seems to progress somewhat along the following lines: The nuclear matter is at first diffuse and may undergo depuration, for in some cases small granules of more deeply staining chromatic matter can be seen near the surrounding cell membrane. The sporont continues to grows till it reaches a diameter of nearly 10μ at which period it becomes evident that the the nucleus has undergone three successive binary divisions, since in medium sized sporonts there are indications of four masses of chromatic matter (pl. 2, fig. 6) while in the largest there are eight readily visible globular bodies (pl. 2, fig. 7). When these ripe sporonts are dissected out, their constricting membrane at first still surrounds the eight contained sporoblasts, for such are the globular bodies within them. Very soon, however, this membrane splits and liberates the small aggregate of sporoblasts (pl. 2, figs. 9 and 10), which now readily stain and show a dense central mass with a strong affinity for haematoxylin. The now detached membrane (fig. 8) is seen to contain a little surplus protoplasm and also often a few grains of chromatic material. Normally, however, this membrane persists till the complete spores are formed, when it dissolves, as I infer from the fact that in none of the sections were there detached membranes among the liberated spores.

Sporoblasts. The mature sporoblast measures about 3μ in diameter. Its nuclear matter could not be differentiated by any of the stains used, but the protoplasm stained deeply with haematoxylin (pl. 2, fig. 10). As the sporoblast matures, a vacuole appears in the center of the protoplasm (fig. 13) and travels towards the periphery; the protoplasm meanwhile becomes more condensed on the side of the vacuole which is farthest from the periphery of the sporoblast. At the same time the cell assumes a more elliptical form and its superficial layer becomes differentiated into a very transparent thick shell.

Spores. The sporoblasts have now been transformed into spores. These are short elliptical bodies, somewhat truncate at the ends, measuring about 3μ x $2.5 - 2.7\mu$ (pl. 2, figs. 14 to 17 and 21 to 24). They still remain attached to one another in aggregates of eight, although they are not in any way actually united with one another. In size and shape they are extremely uniform, though I have observed two larger spores measuring some 8 x 5μ. As the occurrence of such forms is extremely rare I am inclined to consider that their presence is due to some abormality in development. In a fresh state the spores show no differentiation except a small refractive area near one extremity. This is the vacuole. If weak iodine solution be added to the water in which the spores are floating the inner wall of the very thick shell can usually be seen, while in a very few cases a comparatively stout filament, some six times the length of the spore, is extruded from the end of the spore farthest from the vacuole. Although undoubtedly all the spores are capable of protruding this filament I was able to cause them to do so in considerably less than one out of every thousand cases, though I tried many of the usual reagents for the purpose. From a nuclear study I am inclined to believe that the spores were not quite ripe and that this accounted for my inability to extrude the filament in more cases. As winter approached development evidently ceased, and spores dissected out of larvae and kept in water showed no signs of continued development.

For studying the internal structures of the spores the most successful stain used was that of azure II and eosin A. B. as previously described. In the young spore the nucleus occupies the extremity of the cell opposite to that occupied by the vacuole. At first it is rather diffuse but later becomes more concentrated to form a small globular body (pl. 2, fig. 14). Meanwhile the cytoplasm becomes still further condensed till it is drawn away from the walls of the spore around the equatorial region as shown in figure 14, etc. With iron haematoxylin all of this stains very deeply and uniformly as though it were all of a chromatic nature, and it would seem that it must have been to some similar appearance in the spore of Glugea varians Léger that Vaney and Conte ('01) referred when they likened the nucleus of this species to a double T. I was unable to find more than two nuclei in these spores. In the spores of Thelohania giardi Mercier ('08) found five nuclei, and in those of Nosema bombycis Stempell ('09) found four. In some of the specimens there were, however, indications of two darker specks one on each side of the vacuole as shown in figure 22. These would correspond to the shell nuclei of Stempell ('09) but I was unable to distinguish any red coloration. As before stated it is not improbable that the spores were immature, and it is quite possible that the two nuclei I was able to demonstrate undergo a further division before maturation. At any rate, this seemed to be the case in the spores of another species studied. At a later stage the cytoplasm is once more pressed back againt the walls of the spore (pl. 2, figs. 16 and 23). This is due to the formation of the polar capsule within this area. As I never found any evidence of this structure projecting into the vacuole I have adapted the diagram of a ripe spore of N. bombycis as given by Stempell ('09) to the form as shown in pl. 2, figure 23. Full details of its growth could not be seen, since it is entirely surrounded by deeply staining protoplasm. A small depression in the protoplasm appears between the two nuclei, at the end of the spore opposite to the vacuole, which increases in depth until it forms a pear-shaped cavity within the protoplasm. In an end to end view of a spore it is seen that the capsule nearly or quite reaches the vacuole, for by focussing up and down one can see right through the spore (fig. 17). The outline of the capsule and its contained filament with the pore in the shell through which it is ejected, were not seen, nor in stained specimens was the filament ever found attached to a spore as depicted in plate 2, figures 23 and 24. These and accompanying figures of the series are purely diagrammatic. The filament is readily detached from the spore and it is then seen that its base is considerably swollen to a knob-like structure (fig. 25).

The host of Glugea bracteata is the larva of Simulium bracteatum and Simulium hirtipes (?). It infects about 10 per cent of the larvae. I have found this parasite only in the Arnold Arboretum, at Forest Hills, Massachusetts.

From the short descriptions of [Nosema] Glugea simulii by Lutz and Splendore ('04 and '08) it would seem that Glugea bracteata is closely related to the octosporic varieties of this species. Although it is stated that the size and shape of the spores of this form are very variable, the one figured ('08) is almost identical in shape with those found in G. bracteata, with the exception of the filament which, according to the text, is some six times as long as that of the latter species. In a figure of an octosporic pansporoblast, also, it would appear that the pansporoblast membrane is subpersistent, although no statement to this effect is given in the text. From the descriptions of the polysporic varieties, which were not figured, it is evident that, if these all represent the spores of the same species, it must be extremely polymorphic, for the extremes of variation in this species alone have heretofore been considered to be of generic value. Should further study prove conclusively that this is the case several of our now accepted genera will have to be placed in synonomy.

GLUGEA FIBRATA SP. NOV.: (Plate 3)

Macroscopic appearance. In later stages this parasite is present in the body cavity of its host as several large irregular milk white masses, which, as a rule, spread through the entire body though they are most voluminous in the swollen apex of the abdomen (pl. 3, fig. 1).

Microscopic structure. If a portion of one of these white masses be placed in a drop of water under the high power of a microscope it will be seen to consist of several different bodies, the most abundant of which, in later stages, are small oval spores. These spores are all separate and for the greater part nearly uniform in size, measuring about 5.8 to 6.6μ x 3.5μ. Occasionally however one can find a much larger spore measuring 9 to

7.8μ x 4.7 to 5.1μ. These two types of spores are termed 'microspores' and 'macrospores' respectively. If weak iodine solution be added to the water in which they are floating a greatly attenuated filament which measures about 170 to 220μ, or thirty to forty times the length of the spore, is usually projected (pl. 3, fig. 25.).

Life history. This can be determined only from sections about 5μ thick. The early stages have not been found.

Myxosporidium. The parasite at the time of discovery was in a rather advanced state of sporulation. No differentiation into ectoplasm and entoplasm could be distinguished in the myxosporidium, the more central portions of which had for the greater part sporulated. In stained sections there was no sharp differentiation between the developmental stages and the spores as was the case with Glugea bracteata, since the chromatic matter of the former retained the haematoxylin stain. The secretions, then, have the characteristic appearance shown in plate 3, figures 2 and 3. Around the periphery of the myxosporidium the myxoplasm had not been entirely transformed into sporonts but contained numerous small masses of slightly staining granular chromatin. Many of these apparently free nuclei were in a state of division (fig. 4), which appeared to be typically amitotic. Around these nuclei a slight condensation of the myxoplasm could be detected. These condensed areas became globular and were finally invested with a delicate membrane (figs. 5 and 6) until each became a spherical sporont about 5.5μ in diameter.

Sporonts. The nucleus of the sporonts becomes very irregular (pl. 3, fig. 6) and apparently undergoes a form of purification, for in some cases a few chromatin grains are passed out to the surface of the cell where they disintegrate. The nucleus then assumes a more definite form and the sporont continues to grow until it measures about 12μ in diameter, when the nucleus again becomes active and divides. There seem to be two forms of division at this stage. In one the nucleus becomes ring-shaped and this ring draws out as shown in figures 7 and 8. In the other the globular chromatic mass simply divides by amitosis to form two hemispherical masses as shown in figures 10 and 11. Each of the nuclei thus formed redivides. I saw this division only twice and in each case the nuclear matter had evidently assumed a ringlike form before division (fig. 12). It is very difficult to trace the developmental stages beyond this point until the sporoblasts are in process of formation, since all the maturation stages are progressing simultaneously in such a small area that they are practically inseparable, and tracing the individual developmental stages is rendered almost impossible. In the next stage that I was able to distinguish with any certainty, there was evidently a third nuclear division for the much enlarged sporont contained eight distinct nuclei (fig. 13).

Sporoblasts. The wall of the sporont now becomes much thickened and indented between the eight regularly spaced nuclei (pl. 3, fig. 14). This indentation progresses between the nuclei which thus become surrounded by a nearly globular mass of cytoplasm as shown in figure 16, which represents a section of one of these bodies. The walls gradually meet around each of these eight sporoblasts and the aggregate is then separated to form eight free spherical sporoblasts (fig. 17) which measure at first about 5.2μ in diameter but later increase somewhat till they measure about 6.2μ.

Spores. The sporoblasts are transformed into spores by a process apparently similar to that described for G. bracteata, except that in this case the spores are entirely free throughout the whole of their development. The spore is oval in shape, measuring 5.8 to 6.6μ x 3.5μ, and is surrounded by a very thick transparent shell. At the broader end there is a very large vacuole, plainly visible in the fresh spore, while at the smaller end is a much smaller vacuole which cannot be seen in untreated preparations. The nucleus does not differentiate so distinctly in the spore of this species as in G. bracteata, though I found indications of it as a minute body which was often dividing or already divided and situated just above the smaller vacuole (figs. 18 and 19). On one occasion each of the two nuclei formed by the division of this primary nucleus was again undergoing division (fig. 20). By staining deeply with haematoxylin the polar capsule is sometimes distinguishable, projecting through the dense protoplasm into the large vacuole (fig 21). The diagram of the spore can be constructed as shown in figure 26, in which the four nuclei, as yet incompletely divided, are indicated, while the polar capsule, projecting far into the vacuole, contains the greatly elongated coiled filament. This filament, when ejected by the action of iodine, attains the comparatively enormous length of thirty to forty times that of the spore (pl. 3, fig. 25). It should be borne in mind that, as Stempell points out, this filament must, from the manner in which it is ejected, consist of a hollow tube which has to be entirely everted during its emergence from the spore! When it first appears it may be in the form of a loose spiral (fig. 23) but this quickly disappears as the filament soon straightens out. This filament is not so readily separated from the spore as is that of G. bracteata, but in the few cases where it was seen to be detached the basal portion was swollen up into a knob (fig. 24) as in that species. In all the spores in which the filament was protruded it was noticed that the spore contents lost their regularity. I was unable to differentiate the parts, but it seemed that the polar capsule shrank and became attached to one side of the spore, while the protoplasm and nuclei settled down into a small area at the narrow end of the spore (figs. 24 and 25).

Macrospores. These were not numerous among the typical microspores, and appeared to be somewhat abnormal, for the shell was much thinner than that of the microspores (pl. 3, fig. 22). When treated with iodine none of them was seen to eject a filament.

This species of Glugea seems to be related to G. varians Léger ('97). The spores, however, are somewhat smaller and the filament is proportionally about twice as long. Léger and Hagenmüller ('08) state that in G. varians the development of the spores is either octosporic or polysporic. This does not appear to be the case with G. fibrata, for though I found on two occasions 16 sporoblasts adhering together, this did not have the appearance of being a normal condition.

The host of G. fibrata is the larva of Simulium bracteatum and Simulium hirtipes (?). In infests about 5 per cent of the larvae. It was found both in the Arnold Arboretum and in Franklin Park, at Forest Hills, Massachusetts.

GLUGEA MULTISPORA SP. NOV.: (Plate 4)

Macroscopic appearance. In its later stages this parasite is present in the body cavity of its host as small rounded white masses often measuring as much as 1 mm. in diameter. There may be but one or many of these masses scattered irregularly throughout the whole body, which is not greatly swollen by their presence (pl. 9, fig. 1). Where there is only one it is not much larger than the more voluminous masses of a multiple infection, and since it does not then distort the body it is not very readily seen, especially as the skin of the host is not very transparent.

Microscopic structure. If a small quantity of one of these masses be placed in a drop of water under a cover glass it is seen, under a high magnification, to consist of many more or less globular bodies, varying in size from 11.5μ to 30μ. The larger bodies consist of aggregates of numerous small sporoblasts or spores. By gently rolling the cover glass the latter aggregates can be readily broken up and will then be seen to consist of somewhat elongate spores measuring about 4μ x 2.5μ.

Life history. Sections of the parasitic masses stained with iron haematoxylin and orange-G show very beautifully the later developmental stages of this parasite, though, owing to the small quantity of material, I have been unable to obtain as complete a series as I could wish. Plate 4, figures 2 and 3, show the characteristic appearance of sections of the parasite in which the later developmental stages are sharply separated.

Myxosporidium. The parasite at the time of discovery was in a rather advanced state of sporulation. Around the myxosporidium was a very definite membrane, though at this stage of development it was impossible to determine whether this was formed by the parasite or the host. The earliest stages were the sporonts, which occurred round the edges of the mass.

Sporonts. These are rounded bodies measuring some 9μ in diameter. The chromatic substance (fig. 4) is very diffuse and spreads in a network throughout the cell. I did not observe the primary division of the sporont, but it is evident that in later stages the nucleus assumes a more regular form at division, after which it becomes once more irregular. The nucleus divides many times and at each division the protoplasm condenses between the newly formed nuclei to form a fine membrane separating them (pl. 4, figs. 5 to 9). Usually the sporont retains its globular form throughout its entire life but in some instances small irregular masses of dividing cells (fig. 6) were formed, which were too small to be primary sporonts and must therefore have represented a stage in some subsequent division. The numerous cells are so closely packed together as division advances that the exposed surface of each assumes a polygonal, usually hexagonal, form (fig. 9). A section through one of these masses shows that the septa dividing the nuclei do not in all cases completely separate the nuclei with their surrounding protoplasm from each other, since toward the center of the sporont they become less pronounced and finally disappear entirely (fig. 10). It is also seen that in the center there are a few nuclei surrounded by protoplasm, around the edges of which fine membranes are in process of formation. The nuclei are still somewhat irregular though much less so than in the earlier stages of division.

Sporoblasts. When a number of nuclei, varying from about 30 to 60, have been thus formed and surrounded by a membrane, the whole sporont swells and each of the numerous uninuceate sporoblasts into which it has now been transformed assumes a more globular shape. At this period of development neither the haematoxylin nor the orange-G stain is retained, but numerous bodies of very irregular size and form, having the appearance shown in plate 4, figure 11, make their appearance in the myxosporidium. Each sporoblast gradually retains more and more of the haematoxylin till by the time it is transformed into a spore its protoplasm stains very deeply.

Spore. Plate 4, figure 4, represents a section through one of the masses of newly formed spores. There does not appear to be any membrane around this aggregate, but the spores are held together by a quantity of surplus protoplasm which does not stain with haematoxylin as does that of the spore. As will be observed, the spores are not symmetrically arranged but are scattered irregularly throughout the protoplasm which forms the basis of the mass. This irregularity is apparently produced by the swelling of the sporoblasts, for it is at this period of development that the sporont usually loses its globular shape. The elliptical spore measures about 4μ x 2.5μ and is less pronouncedly ovoid than that of Glugea fibrata. On treatment with iodine a filament is ejected. Unfortunately I have no preparations showing the evaginated filament, but in a free-hand sketch of an unstained specimen I drew it about ten to fifteen times the length of the spore and this is roughly its length. My preparations were also stained with haematoxylin so I was unable to see the nuclei, but in figure 14 I have, by analogy with G. fibrata indicated their probable position. I observed in unstained specimens that when the filament was ejected by iodine the spore contents shrank in a similar manner to that described for G. fibrata, giving the spore the appearance of figure 13.

It is seen from this that the ejection of the filament in both of these species is accompanied by a decided enlargement of the vacuole, which suggests that its fluid contents are swollen by the iodine entering through the shell by osmosis, and that the as yet undiscovered function of the vacuole may have some connection with the ejection of the filament.

The host of G. multispora is the larva of Simulium vittatum and Simulium bracteatum. It was found in one out of four larvae taken from a part of the stream in the Arnold Arboretum, where larvae were very scarce. Three other infested specimens were taken at Hyde Park and Franklin Park in the neighborhood of Boston.

NOTES ON THE GENERIC POSITION OF THE SPECIES DESCRIBED

Although I have placed all three species above described in the Genus Glugea of Thélohan, the first two, especially G. bracteata, according to Gurley's interpretation of the genus ('94), cannot be included, for his definition of the genus is as follows. "Glugeidae possessing a myxosporidium, and in which the pansporoblast produces *an inconstant but large number* (*always more than 8*) *of spores, pansporoblast membrane not subpersistent.*"[3] In

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[3] Italics mine.

G. bracteata and G. fibrata the pansporoblasts produce regularly eight spores and in the former case the pansporoblast membrane is subpersistent. This latter type of sporulation is characteristic of the genus Thelohania of Henneguy, but in this genus the meronts remain separate, are uninucleate, and each gives rise to one pansporoblast. G. bracteata therefore cannot be placed in this genus, neither can it be included in Pleistophora of Gurley, in which also the pansporoblast is multisporulate (always more than octosporulate), although its membrane is "subpersistent as a polysporophorus vesicle." This species must therefore either be placed in the genus Glugea, in which case Gurley's definition must be modified, or a new genus will have to be made to include it. The peculiar shape of the spores and the subpersistence of the membrane might justify the latter course, but since Lutz and Splendore ('04 and '08) have pointed out that in the same species the pansporoblasts may be octo- or polysporic, and since according to the interpretation of Minchen ('03) and Stempell ('09), this species can be included in the genus Glugea, I am placing it provisionally there.

I am convinced that the species above described are closely related to the South American microsporidian described by Lutz and Splendore ('08) as Nosema simulii. These authors ('04) also place the species varians of Léger in the same genus. The reason for transferring this species is based mainly upon the fact that, as these authors show, the number of spores formed in a pansporoblast (i.e., whether octo- or polysporic) is an unreliable generic character. Nosema, however, has no myxosporidium and maturation takes place by its numerous uninucleate and separate meronts giving rise directly to spores, thus omitting a sporont and sporoblast stage. The absence of a myxosporidium places this genus in the family Nosematidae. That simulii does not belong to this family is shown by the fact that the myxosporidium is present as "rounded cysts, with fine membranes, in which are contained thin walled secondary cysts" (pansporoblasts). This character places this species, as well as varians, and those above described, with either the Pleistophoridae or the Glugeidae, which are separated by having multicellular, or multinuclear unicellular myxosporidia respectively during the period of growth. This character disappears when sporulation begins, for in both families the sporonts are surrounded with a fine membrane, and the myxosporidium therefore at this stage is multicellular in both families. As before stated, all the material I have examined was in rather an advanced stage of sporulation, so that this character was poorly defined. As the earlier stages were not described by Léger ('97) or by Lutz and Splendore ('04), I conclude that their material was in about the same stage of maturation as that which I examined. In Glugea fibrata, however, I saw (pl. 3, figs. 4 and 5) what appeared to me to be free nuclei in the protoplasmic mass between the already formed sporonts. Some of these seemed to be still undergoing division. In G. bracteata also, sporonts appeared to be budded off from a multinucleate mass of protoplasm (pl. 2, fig. 4). If my interpretation be correct, both of these species certainly, and probably the other three also, belong to the family Glugeidae, and must therefore, as before stated, be provisionally placed in the genus Glugea. If this is not the case and the myxosporidium is multicellular during its entire development, these species will have to be placed in the Pleistophoridae, and, since Lutz and Splendore ('08) show that octo- and polysporic development is not of generic value, all can be included in a modified conception of the genus Pleistophora Gurley, though that author limited the genus to polysporic, i.e., more than octosporic, species.

THE EARLY STAGES OF INFECTION

As stated in the detailed account of the various species given above, the early stages of infection and development were not found, and if, as I infer, they are present only in the youngest Simulium larvae, it is unlikely that these incipient stages will be readily discovered. From analogy and from observations upon the later stages it is, however, possible to trace the probable means of infection and subsequent development.

As in all other Myxosoporidia, it may be assumed that infection is effected by way of the alimentary tract, and it is almost safe to say that this infection can take place only in the mesenteron, for the stomenteron and proctenteron are rather heavily lined with chitin, which would repel any attacks of the unarmed germ which escapes from the spore. In order that the filament may be expelled previous to the escape of the germ it is necessary that the spore be acted upon by some dehydrating, or similar reagent, such as is found in the digestive juices of its host. Bütschli ('82) kept spores for a long time in water and noticed that there was no change in them. This is also the case with the spores from Simulium larvae which I kept for two months in water, at the end of which period they had undergone no change. Rare instances of spores dehiscing while still in the body of the original host have been described by Lieberkühn ('54) and Simond ('03), but these are exceptional.

The germ, then, is liberated in the mesenteron of a young larva. If, according to the theory explained above, the peritrophic membrane has not been completely formed at this time, it is able to come in direct contact with the mesenteric epithelium and to work its way in amoeboid fashion between the cells till it escapes into the body cavity of its host. If, on the other hand, the peritrophic membrane already completely lines the entire mesenteron and proctenteron it seems that the minute germ must pass straight through the intestines and be voided with the faeces. In this way I would explain, as before stated, the absence of any indications of parasitisation being effected in any but the earliest stages of larval life.

The young germ, after entering the body cavity, lives freely in the blood plasma, but does not, probably, multiply by division as does that of N. bombycis Näg. (Stempell '09), and where several myxosporidia are present in one host these must, it would seem, be considered as separate infections, except in the case of Glugea multispora in which so many myxosporidia sometimes occur that it would seem to indicate some such multiplication. The germ attacks a cell of the fat body. Evidence of this is shown in many cases where the walls of these cells are seen still surrounding small irregularities of the myxosporidium (pl. 2, fig. 3). Here the germ loses its motility and a constant (binary?) division of the nucleus begins, which continues throughout the entire meront period, at the end of which the myxosporidium has grown to a very great size and consists of a multinucleate protoplasmic mass measuring up to 2 to 3 mm. in length. Each nucleus in the center of the myxosporidium now collects around itself a small mass of protoplasm, which becomes somewhat denser and is finally enveloped by a membrane, thus forming a globular unincleate sporont as previously described. This action spreads from the center outwards until the whole myxosporidium has been converted into sporonts, although by this time those in the center have been already transformed into spores.

When several myxosporidia are present in one host they all remain small so that they together occupy only as much space as a single fully developed myxosporidium. The reduction in size does not, however, interfere with development, for the small trophozoites begin to sporulate at the same time as do those which are fully grown. It seems, moreover, that the life history of the parasite closely coincides with that of its host, for in every case where sporulation was nearly complete, its host was full grown and the surrounding larvae from the same batch of eggs were pupating. In northern latitudes Simuliidae pass the winter in the larval stage and at about the end of October development practically ceases. It appears that the parasites in such larvae likewise cease to develop, for in all infected larvae collected during November and December the parasites were in precisely the same condition of development. This cessation of sporulation during the winter was noticed also by Cohn ('96) in a species of Myxidium.

THE EFFECTS OF THE PARASITE ON THE HOST

I was not able to find that any organs except the fat body are attacked by these parasites. The musculature, spinning glands, and epithelial cells of the alimentary tract showed no signs of infection. I have not, however, been able to find the reproductive organs in any parasitised larvae. These are always small, but can as a rule be found in a good series of sections from a healthy larva. When the parasite, however, is present no trace of these organs can be seen.

Pérez ('06) found, that in the case of crabs, the presence of Thelohania sp. in the ovary caused the parent to reabsorb its eggs. There are no indications in Simuliid larvae that the reproductive organs are actually infected. Since they are absent when but one myxosporidium is present, which may be situated ventrally to the alimentary tract, it seems more probable that, as in the case of Trichonympha spp. in Termites, the presence of the parasites causes suppression in the development of the reproductive organs without actually coming into contact with them.

In no preparations was there any noticeable hypertrophy of the alimentary tract, as seen by Léger ('97) in S. ornatum when parasitised by G. varians Léger or by myself ('11) in S. hirtipes when parasitized by various Myxosporidians. There was, however, a varying effect upon the histoblasts, from an almost complete atrophy to hardly any noticeable reduction in size (pl. 6, fig. 6). For a fuller account of the histoblasts, and the effects of the parasites upon them, I would refer the reader to my former paper ('11).

From numerous observations, I feel convinced that parasitised larvae never pass through the pupal to the adult stage. Unfortunately, owing to the difficulty of keeping these larvae alive under artificial conditions, I am unable to make this statement with absolute certainty, especially as evidence goes to show that in the infection of allied insect larvae by Glugeid parasites the host does not always suffer. In all of the latter cases, however, the parasite belongs to the family Nosematidae. The following is a brief account of two interesting species of this family.

In 1903 Simond described a Nosema sp. parasite of the larva of the yellow fever mosquito, Stegomyia fasciata Theob. from Rio de Janeiro, in which he found two distinct types of spores. The most numerous spore was unicolored and measured 3 to 5μ x 2 to 3μ. These, he states, dehisced within the host and thus caused auto-infection. This is the only case in which regular auto-infection has been described. Less frequently he found brown spores, which were less symmetrical in form and gave rise to an attenuated brown filament, which was occasionally branched. This attained a length of 20 to 30μ, when it assumed a necklace-like form, after which it disintegrated without apparent further development or function. The infected larvae pupated and the parasite was present in the adult. In 1908 Lutz and Splendore also described briefly a species from the adults of Stegomyia fasciata which they named Nosema stegomyiae. The size of the spore was approximately that given by Simond and it is quite possible that this is the same species.

Hesse ('04) described a species, Thelohania legeri, parasitic in the larvae of the malarial mosquito, Anopheles maculipennis Meig. The fat body of these larvae was the seat of infection, as in the case of Simulium. There were two types of spores present, viz., microspores, 8 x 4μ, and macrospores, 12 x 5μ. The filament was readily evaginated by the use of iodine. Although he did not find the parasites present in any adults he states that it is doubtless to be found in this stage, for the host does not suffer in any way from the effects of the parasite.

In these two cases the parasites were not, as far as the observations show, in any way detrimental to their host. It must, however, be borne in mind that neither Nosema nor Thelohania, which are both genera of the family Nosematidae, form such large masses of parasitic material as Glugea, and that in the descriptions of these two cases there is no mention of the parasite causing the body of its host to be in any way distended. This fact would surely not have been omitted had the effect been as marked as in the case of the Simulium larvae.

In the parts of the stream where the parasites were most abundant I collected all the pupae I could find. These were sectioned, or dissected, but in no case could signs of any stage of the parasite be detected. When healthy and parasitised larvae were brought together into the laboratory the healthy larvae always lived for a much longer time than the parasitised individuals, and in several of the latter death was apparently caused by the skin rupturing, when the mass of parasitic material protruded through the rent thus made.

Mature larvae turn brown a little before pupation and the histoblasts of the pupal respiratory filaments blacken (pl. 6, fig.

5, *r.f.*). In no cases of parasitised larvae was this stage of maturation observed, and it is probably seldom, or never, reached.

In comparing sections of healthy and parasitised larvae, it is seen that in the latter the quantity of fat-body stored up for the formation of adult tissues is greatly reduced, and is probably entirely inadequate for the requirements of a full-sized fly. It is also evident that, owing to the large quantity of parasitic material, which is of a somewhat firm consistency, a large rent in the skin of the host would be necessary, in order to liberate it, and this would certainly cause the death of the host. Such was observed to be the case in larvae which died in this way when in captivity. On the other hand, should the parasite pass over into the adult, it is inconceivable how the fly, already weakened by the diminution of its fat body, could ever get out of the water when handicapped with a solid mass of spores which would swell its abdomen to quite three times its normal size. Taking into account all these facts, which in brief are the absence of mature larvae containing parasites, the absence of parasites in the pupae, the suppression of the reproductive organs, and often of the histoblasts, the voluminous proportions of the parasite and the resulting restriction of fat-body, I feel justified in stating that in almost if not all instances, the presence of Glugeid parasites prevents the maturation of their larval host.

A GREGARINE PARASITE OF SIMULIUM LARVAE: (Plate 5)

During early October and later it was found that, in two of the streams inhabited by Simulium bracteatum, a number of the larvae were somewhat enlarged and had generally a lighter color than the average individuals. Closer inspection showed that they were heavily parasitised by innumerable small cysts measuring up to 0.25 mm. (pl. 5, fig. 1). A number of these larvae were collected, killed and sectioned, when it was seen that these cysts affected various tissues of the body. The greater number of them were already free, floating in the blood plasma, but those which were still retained at the point where they began growth, were situated in the epithelial cells of the integument (pl. 5, fig. 2), in the cells of the fat body (fig. 3) and in the pigment cells which cover the nervous system (fig. 4). The sexual organs were never found and it is probable that they had been parasitised and destroyed. I saw no signs of the epithelial cells of the intestine, spinning glands or Malpighian tubules suffering, neither did it appear that the muscles were ever affected. Under a higher magnification the cysts were seen to consist of a grandular cytoplasm containing small, irregularly distributed masses of chromatic material (fig. 5). In some young cysts there were vacuoles, but these were only detected in living specimens. In other fresh material there seemed to be a distinct ectosarc layer of a perfectly clear fluid. Otherwise the contents of the cyst seemed to be quite homogeneous and to consist of a granular protoplasm. On treating with osmic acid this turned a deep brown, indicating the presence of fat. By the end of November I noticed that the protoplasm was beginning to collect around the chromatic masses and the cell contents were divided up into many multinucleate irregular bodies (pl. 5, fig. 6). During December these bodies split into uninuclear, nearly globular bodies (fig. 7). If a cyst was then dissected and allowed to float in a drop of water it soon burst, liberating countless numbers of these minute globules. After they had been liberated about a quarter of an hour they began to move independently. Soon they became very active, though their power of locomotion was very slight, for they did not move their relative position appreciably but darted back and forth over a limited area. Each was provided with a flagellum. The actual movement could not be accurately observed as all motion ceased as soon as the specimens were placed under a cover glass in a cell slide. I sent prepared specimens of these parasites to Professor Calkins who very kindly replied that they probably belonged to the order Gregarinida. I have not, however, sufficient stages to be certain of this, but if this be the case there are one or two characters which are not quite in accordance with those usually connected with Gregarinida. In the first place it is evident that this parasite increases by schizogeny in its early stages, for in every host in which it was found the number of cysts present was estimated to be between 500 and 1000 or in some cases more, while surrounding larvae of the same generation were free from attack. Secondly, the nucleus is not single from the earliest observed stages, but seems to occur for the greater part of the organism's life in the form of diffusely scattered chromatic masses. There is, again, usually no sharply defined layer of ectoplasm surrounding the endoplasm, while in the latter vacuoles are present. Another feature is that the presence of this parasite has a marked pathological effect, for parasitised larvae have the development of their histoblasts arrested (pl. 6, fig. 7) so that they must die at, if not before, pupation. Since in the streams in which this parasite is present it must kill well over 50 per cent of the somewhat scarce larvae, it would seem that it must be an important agent in the reduction of Simulium larvae in the neighborhoods where it occurs, and for this reason, apart from its scientific interest it would be of advantage to ascertain in detail its life history and distribution.

The host of this Gregarine is the larva Simulium bracteatum. It was found infecting about half of the larvae in streams in Franklin Park and at Hyde Park near Boston, Massachusetts, from October to December. The streams where it occurred had not been examined earlier in the year and contained no species except S. bracteatum.

THE ECONOMIC VALUE OF PARASITES OF SIMULIIDAE

It will be seen from the foregoing notes that there are in the neighborhood of Boston three distinct classes of parasites infecting, and in each case killing, Simulium species in their larval stages. Summarizing my observations for the single year, 1911, there are the following:

1. Parasites of the spring brood of Simulium.
a. Various Myxosporidia *sens. lat.*, up to 80 per cent mortality
c. Mermis sp., up to 25 per cent mortality
II. Parasites of the fall brood of Simulium.
a. Glugea bracteata, about 10 per cent mortality
a. Glugea fibrata, about 5 per cent mortality
a. Glugea multispora, rare
b. Gregarine species, up to 50 per cent mortality

From no other locality in North America have these, or similar parasites, been recorded. As mentioned earlier in this paper, Simulium larvae have been carefully and extensively studied in several sections of the country, notably at Ithaca, New York, and in Maine, but in none of these places has a single case of parasitism been recorded. It is evident, as will be seen from the illustrations of parasitised larvae, that such individuals could hardly escape the eye of the observer, especially as, in streams in which infection is heavy, the greatly distended and whitened larvae can be readily seen from a distance of several yards from the water. We thus have evidence, though by no means conclusive, that these parasites are not widely distributed throughout the United States. Other evidence is found in the fact that in this neighborhood the black flies are not a serious pest, although the nature of the country is eminently suited to the requirements of their larval life. In surrounding states, in every direction, these flies are not only a great nuisance, but also a dangerous pest. The southern states, especially, suffer from these blood-thirsty flies. In the interesting report of Professor Lugger, of the University of Minnesota ('96), it is stated that in the State of Tennessee alone, these flies were responsible, in the year 1892, for the loss of $500,000, through their attacks on cattle. But their ravages are not confined to the southern states, for in the northern state of Maine, and in New Brunswick, one hears of the death of animals due to the attacks of these flies, while in the same localities it is impossible at certain seasons of the year to enter forests, unprotected, in the neighborhood of streams. It would therefore seem that we owe our deliverance largely, if not entirely, to the fact that a large percentage of these flies is annually killed off before maturity.

I have made no experiments upon the transference of these parasites from one Simulium species to another, but, as far as can be seen, there should be no difficulty in accomplishing this, for in all cases observed *the parasites infected all species of larvae, present at that time, in the stream where the former occurred.* There is, however, a seasonal variation of parasitism, for the species found in the spring were not retaken in the fall, and vice versa, so that it is probable that only those species of Simulium, whose life history coincides with parastised species, could be infected with the parasites of the latter. Since, as previously stated, I am led to believe that infection is possible only during the early stages of larval life, it would probably be necessary to collect a number of infected larvae, and place them in streams where the other species is always abundant, some little while before the eggs of the latter hatch. If the parasite is present in the larvae of the following generation it will be readily detected during the later stages of larval development.

It will be seen from the list of Myxosporidia, and the seasons in which they were found, that those occurring in the spring brood parasitise a much higher percentage of larvae than those occurring in the fall brood. This is due, it seems, to the much greater numbers of larvae present in the streams during the former season, for at this time they are living a gregarious life, and all stages of development are present together. This results in the newly hatched larvae always being in close proximity to some recently dead larva, from which innumerable spores have escaped. In the fall, however, larvae are comparatively rare, and are more solitary in their habits, so that the infection of young larvae is less certain.

The Gregarine species, however, which occurred in two streams, not examined in the spring, was found heavily to parasitise larvae which were not by any means abundant in the streams, and this is probably due to the motility of the bodies liberated by the host upon its death. It would thus seem that, were the parasites liberated in streams where Simulium larvae are very numerous, the spread of infection would be rapid, and the disease should soon be firmly established.

It is not easy to understand how it is that the parasites do not slowly travel down-stream, from year to year, so that in time the stream becomes clear of infection. That this is not the case, is indicated by the fact that where the source of a stream has been found as a spring, Simulium larvae occurring near this region were found to be parasitised. As before stated, I do not believe that the adults are capable of spreading the infection through the ova. Krassilstschik ('96) found that the spores of N. bombycis (pébrine) could pass through the intestines of birds and be still capable of infecting silkworms. This may also be the case with these spores, since many must be ingested by birds which drink at the streams. Other insects, frogs, etc., may also carry the spores upstream. Perhaps also, Simulium larvae themselves move about more than is generally believed, and as stated in my previous paper ('11), parasitised larvae are more active than healthy specimens. It may be, therefore, that such larvae occasionally travel a considerable distance up stream before death, and thus prevent the disease from being washed out of the stream.

I found no definite signs of Mermis during the fall of 1911 although, in one or two dissections of S. bracteatum, I found minute worms, measuring about 20μ among the masses of G. bracteata contained in these larvae. This Mermis should also prove of good economic value, since there can be little doubt that it is a general parasite which could be readily transferred from one species to another.

SUMMARY

Although the country in the vicinity of Boston is eminently adapted to the breeding requirements of black flies (Simuliidae), they do not occur in sufficient abundance in this neighborhood to constitute a dangerous pest, or even a serious annoyance. Black flies or 'buffalo gnats,' are most destructive in the southern states, as an example of which Tennessee may be cited, where in one year the cattle-raisers suffered a loss of $500,000 through the attacks of these insects. Their ravages are not, however, confined to the southern states, for in the more northern states, as in Maine and Wisconsin, these flies, at certain seasons of the year, are extremely abundant, and are very injurious to stock. It would thus seem that in the neighborhood above named the comparative freedom from annoyance by these small, though vicious, flies must be due to some other cause than climatic conditions. An examination of the larvae in the small streams, which occur so frequently in eastern Massachusetts throws some light on this phenomenon, for it is seen that, in many cases, a large percentage of the insects contain parasites that are in every case fatal to their host. The Simuliidae occurring in this neighborhood are most abundant in the spring, and it is at this season that parasitism is most effective. The most common species is Simulium hirtipes. This is found to be heavily parasitized with species of Myxosporidia (*sens. lat.*). Sometimes as many as 80 per cent of the larvae are found to be greatly distended with masses of this white parasite, which in every case spells death to its host. Another spring parasite is a Nemathelmith belonging to, or near, the genus Mermis. The presence of this, also, is fatal to the Simulium larva in which it lives, and by this means some 25 per cent of the larvae in certain streams were destroyed during the spring of 1911. Very few Simuliidae are to be found in the streams throughout the summer, but in the fall the early stages of S. bracteatum are common. The hitherto undescribed pupa of this species is of interest in that the respiratory filaments are only four-branched, a condition previously not recorded from North American species, and only twice found elsewhere. Together with these larvae occur a few of those of S. vittatum, and later in the season S. hirtipes is once more in evidence. All of these suffer from Microsporidian parasites, which do not confine their attacks to any one species of Simulium larva, but freely parasitise all species present in the streams during the season of their occurrence. Three Microsporidian species have been found, and their later developmental stages have been traced with sufficient detail to determine their probable taxonomic position.

Since no accounts of Microsporidia, and their relation to insects have been made in this country, it was deemed advisable to give a somewhat detailed account of this suborder of the Sporozoa.

The three species described from Simulium larvae are:

I. *Glugea bracteata sp. nov.* Pansporoblast octo-sporulate. Pansporoblast membrane subpersistent. Spores about $3\mu \times 2.5\mu$, short elliptical, filament about six times the length of the spore.

II. *Glugea fibrata sp. nov.* Pansporoblast octosporulate. Pansporoblast membrane not subpersistent. Spores about $6\mu \times 3.5\mu$

ovoid, filament about thirty to forty times the length of the spore.

III. *Glugea multispora sp. nov.* Pansporoblast polysporulate. Pansporoblast membrane not subpersistent. Spores about 4μ x 2.5μ.

The earliest stages were never seen. It is conjectured, however, that the parasite, which undoubtedly enters the body cavity through the alimentary tract, is able to parasitise only young larvae. This is thought to be due to the postembryonic development of the cephalic fans and peritrophic membrane, both of which are highly specialized organs and in Simulium larvae show unique and interesting modifications. The parasite was found attacking only the cells of the fat-body. These are soon ruptured and the parasite subsequently leads a free life in the body cavity of its host, where it grows to comparatively enormous volume, consisting of a multinucleate mass of protoplasm which greatly distends the body of its host. As the latter matures, the myxosporidium, as the parasite is now termed, is converted into innumerable thick-shelled spores. These escape from the larva, which has succumbed to the attack, and by entering the alimentary tract of fresh larvae spread the disease.

Since these parasites are to be found in all species of Simulium larvae present in the streams where they occur, it is probable that they could be transferred to fresh streams and thus cause the infection of species of Simulium larvae in which as yet no parasites have been found. The parasite rarely, if ever, passes over into the adult, so that the natural spread of the disease from stream to stream is slow. That these Microsporidians are not widely distributed throughout the United States is indicated by the fact that though Simulium larvae have been carefully studied in several localities no cases of their presence have been recorded. When infected larvae are at all abundant in streams, their greatly distended and whitened abdomens are very conspicuous, and are readily seen at some distance from the water, under conditions in which normal healthy larvae are almost or quite invisible.

A further parasite in the fall generation, probably a Gregarine, has been found, infecting in some streams as many as half of the larvae of S. bracteatum, which was the only species present in those parts of the streams where it occurred. Its life history has not received careful attention, but it is evident that it is fatal to its host, and it produces if anything more 'spores' than do the Microsporidia. This parasite, therefore, deserves careful study, for though it was found only in one species of Simulium, this was probably due to the fact that no other species was present where it occurred.

It is hoped that economic entomologists will, in future, look for the presence of these various parasites. If it is then found that those districts in which Simulium is not a serious pest, owe their deliverance to the presence of these parasites, experiments can be undertaken to prove whether the latter can be artificially propagated. If this be feasible, and the writer sees no reason to doubt it, there is a prospect that in those districts where black flies are most numerous, the diseases would spread with great rapidity and considerably reduce the devastations and annoyance caused by these little Diptera.

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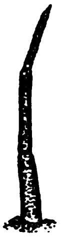
PLATE I

EXPLANATION OF FIGURES

Larva and pupa of Simulium bracteatum Coq.

1 Larval antenna × 260
2 Head capsule of recently moulted larva to show the characteristic markings, × 32.
3 Mandible of larva, × 260.
4 Hypopharynx of larva, × 260.
5 Maxilla of larva, × 260.
6 Labium of larva, × 260.
7 Labial teeth, × 1040.
8 Pupa in cocoon, × 20.
9 Characteristic branching of respiratory tubes × 40.

PLATE 1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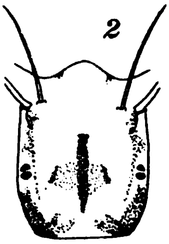
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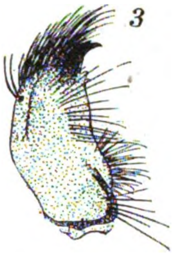
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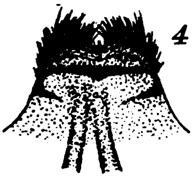
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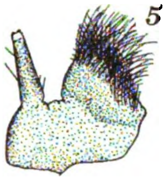
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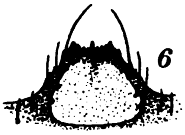
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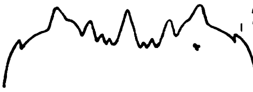
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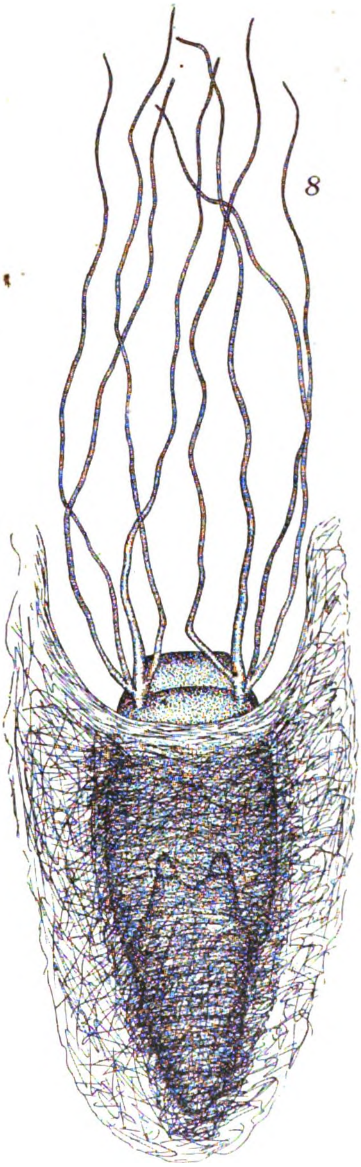
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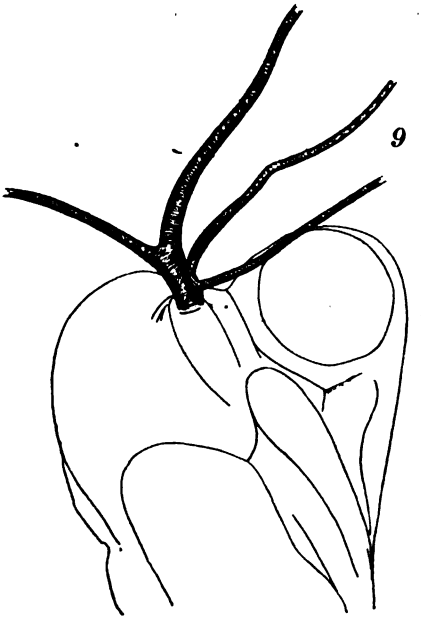
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PLATE 2

EXPLANATION OF FIGURES

Glugea braceata sp. nov.

1 Parasite in situ, × 12.

2 Section through abdomen containing parasites, × 12.

3 Section through sporulating Myxosporidium, × 470; *f.*, fat body tissues still attached.

4 Portion of Myxosporidium showing budding of sporonts, × 470.

5 A newly formed sporont, × 1400.

6 Sporoblasts in process of formation, within sporont, × 1400.

7 Pansporoblast = Sporont containing 8 complete sporoblasts, × 1400.

8 Empty shell of pansporoblast with a little chromatic material and surplus cytoplasm still adhering, × 1400.

9 and 10 Aggregate of sporoblasts, stained with haematoxylin and orange-G, × 1400.

11 and 12 Aggregates of spores unstained × 1400.

13 to 16 Maturation of the spore, stained with azure and eosin, × 1400.

17 End to end view of a mature spore, × 1400.

18 Mature spore, unstained, with filament ejected, × 1400.

19 and 20 Diagrammatic representation of maturing sporoblast to show the formation of the vacuole, × 7000.

21 and 22 Diagram of maturation of the spore, nucleus dividing and cytoplasm becoming more dense, × 7000.

23 Diagram of situation of polar capsule, with enclosed filament, × 7000.

24 Diagram of spore with filament ejected, × 7000.

25 The swollen knob at the base of the filament.

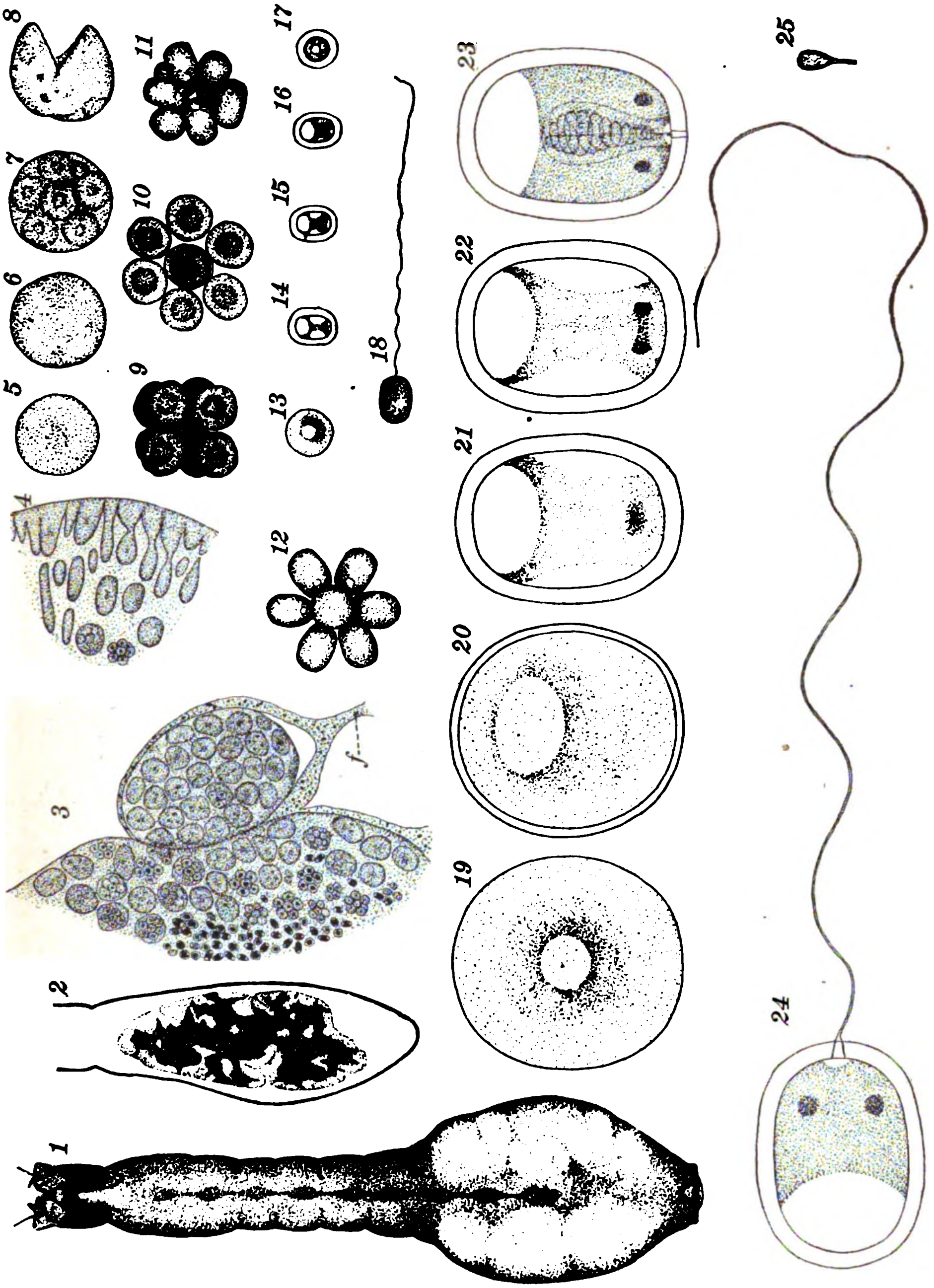
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PLATE 3

EXPLANATION OF FIGURES

Glugea fibrata sp. nov.

1 Parsites in situ, × 12.
2 Section through sporulating Myxosporidium, × 470.
3 Section through abdomen containing parasitic mass, × 12.
4 and 5 Dividing nuclei of meronts, × 1400.
6 Newly formed sporont with scattered chromatic matter, × 1400.
7 to 11 Division figures of sporont nuclei, × 1400.
12 Second division of nuclei in sporont, × 1400.
13 Sporont containing 8 nuclei, × 1400.
14 Protoplasm collecting round each nucleus to form 8 sporoblasts, × 1400.
15 Sporoblasts nearly complete, × 1400.
16 Section through 15, × 1400.
17 Free sporoblast, × 1400.
18 to 20 Maturation of the spore, stained with azure and eosin, × 1600.
21 Spore deeply stained with haematoxylin to show the polar capsule, × 1600.
22 A macrospore, × 1600.
23 Spore with filament partially ejected, × 1600.
24 Spore with filament detached, × 1600.
25 Spore with fully extended filament, up to 40 times its length, × 1600.
26 Diagram of mature spore, adapted from W. Stempell, × about 5700.

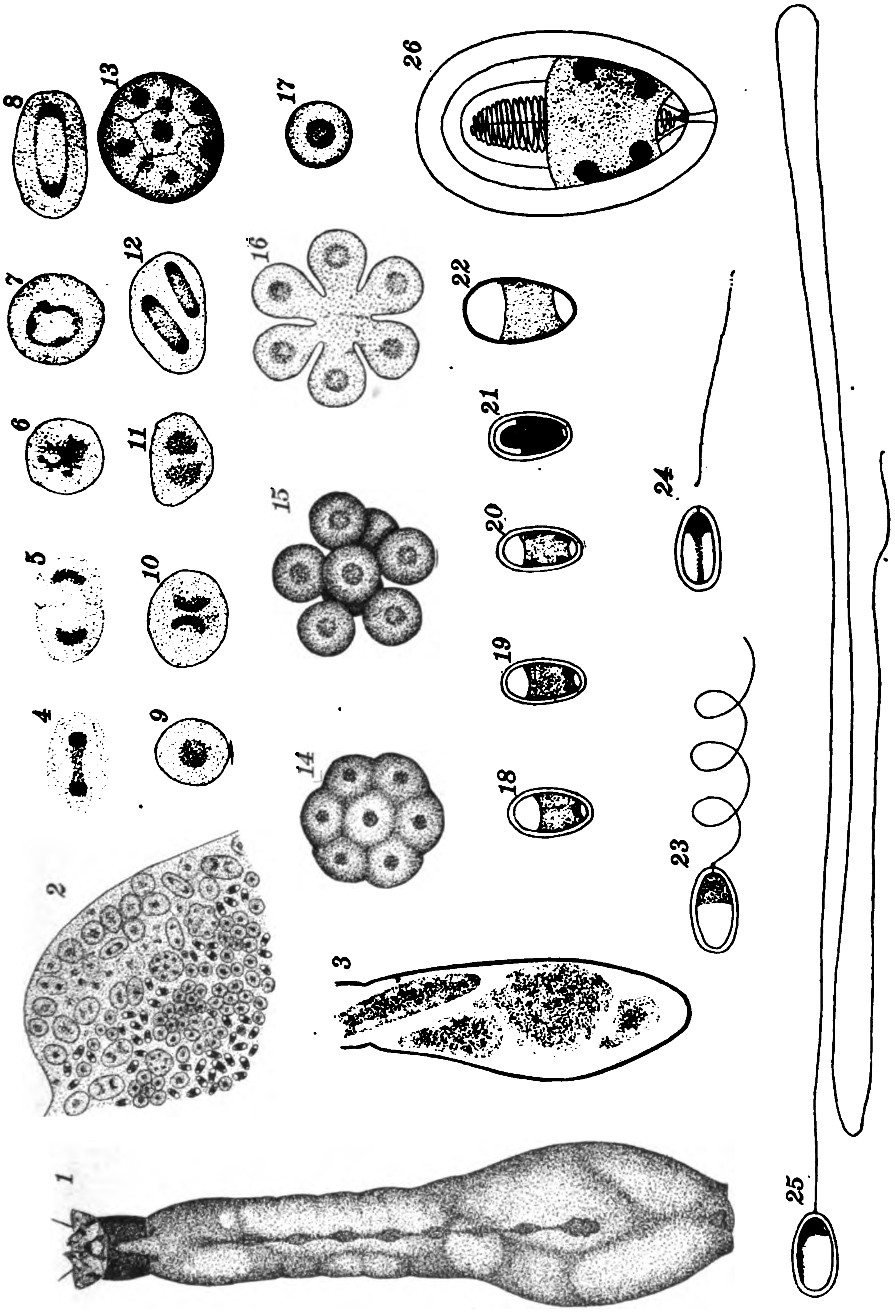
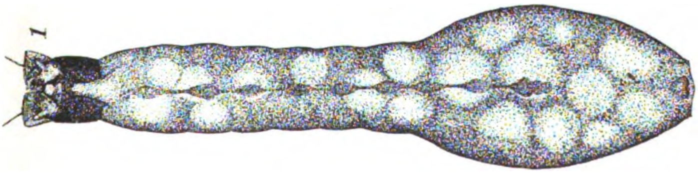


PLATE 4

EXPLANATION OF FIGURES

Glugea multispora sp. nov.

1 Parasites in situ, × 12.
2 Section through sporulating myxosporidium, × 470.
3 Section through abdomen containing parasite, × 12.
4 A young sporont, × 1400.
5 to 9 Division of sporont into sporoblasts, stained with iron haematoxylin and orange G, × 1400.
10 A section through 9, × 1400.
11 Pansporoblast unstained, × 1400.
12 Section through mass of spores, × 1400.
13 Spore with filament ejected, × 1400.
14 A diagram of a spore, × 7000.



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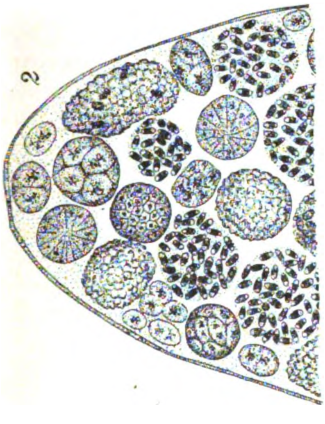

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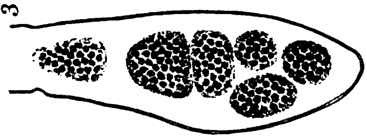

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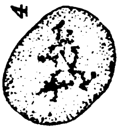

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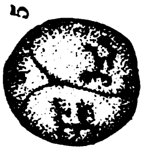

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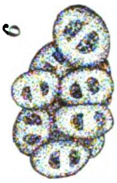

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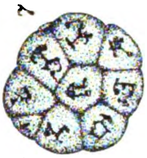

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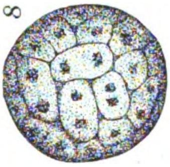

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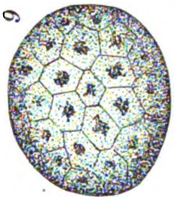

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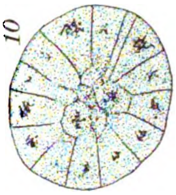

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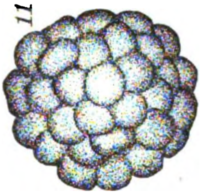

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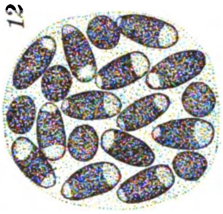

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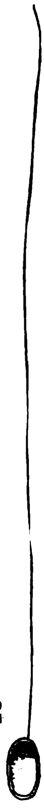

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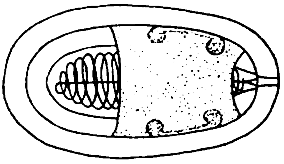

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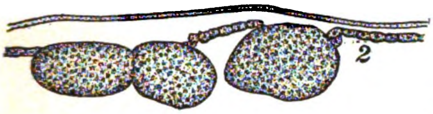
PLATE 5

EXPLANATION OF FIGURES

Gregarine parasite of Simulium bracteaum

1 Parasites in situ, × 20.
2 Cysts from integumental epithelium, × 170.
3 Cysts in cells of the fat-body, × 170.
4 Cysts from pigment cells covering the nerve ganglia, × 170.
5 Portion of a cyst at an early stage, × 1400.
6 'Sporulating' cyst, × 1400.
7 'Spores' in a cyst, almost mature, × 1400.
8 Flagellate 'body' from a cyst, × 1400.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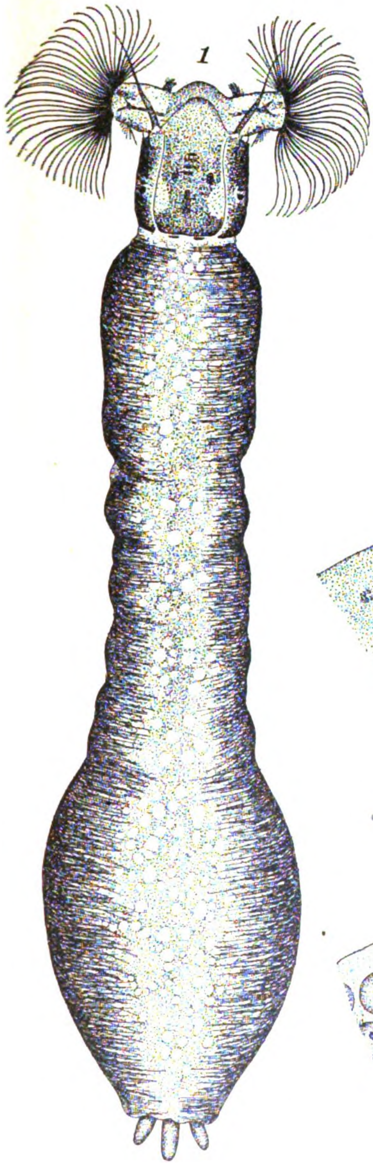
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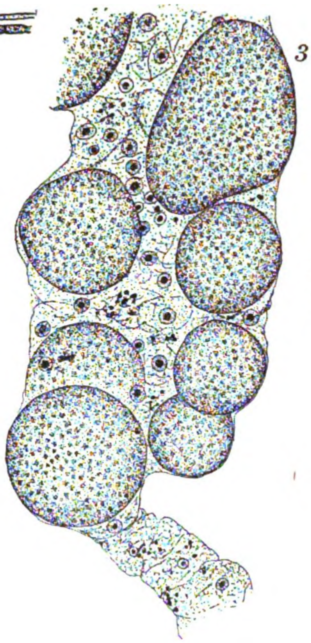
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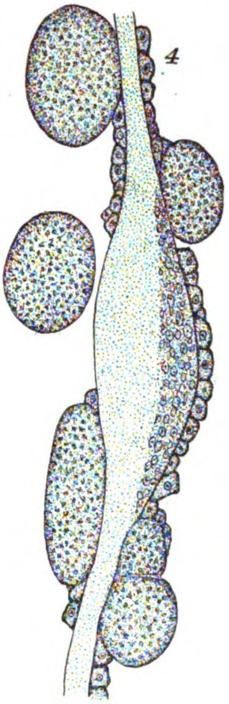
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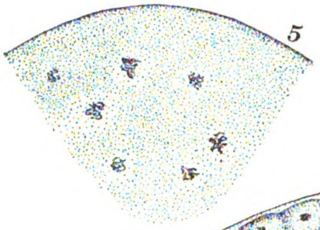
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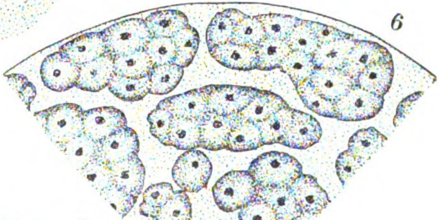
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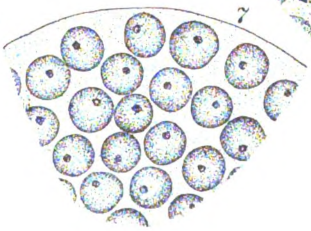
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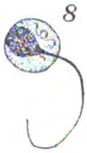
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PLATE 6

EXPLANATION OF FIGURES

1, 2, and 3 Types of flagellate bodies from Gregarine cyst, × 1400.

4 Most common type of flagellate body, × 2200.

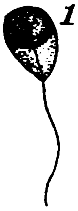
5 Diagram of histoblasts of mature healthy Simulium larva; *r.f.*, respiratory filament (pupal) histoblast; *w*, wing histoblast; *h*, halter histoblast; 1b, 2b, 3b, leg histoblasts.

6 Diagram of histoblasts of full grown Simulium larva containing Microsporidia, lettering as in fig. 5.

7 Diagram of histoblasts of full grown Simulium larva containing 'Gregarine;' lettering as in fig. 5.

8 Longitudinal section through one half of the proventriculus and cardia (see text figure, page 00, for explanation).

9 Diagram of a section of the mesenteron. *p.m.*, peritrophic membrane; *c.m.*, circular muscles; *l.m.*, longitudinal muscles.



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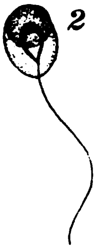

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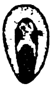

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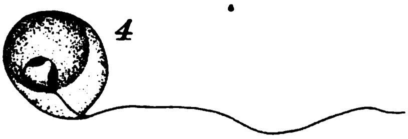

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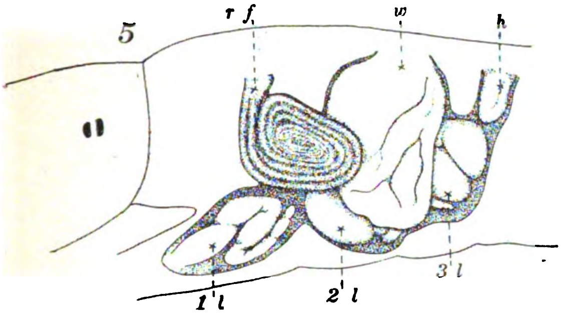

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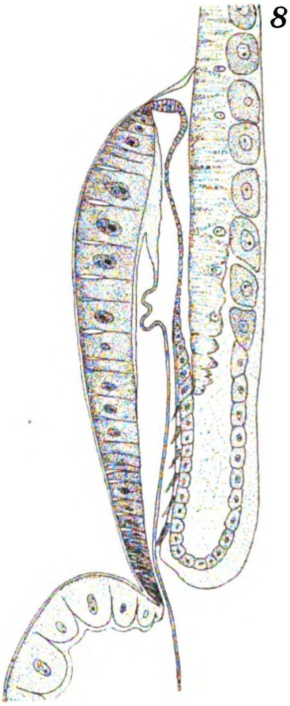

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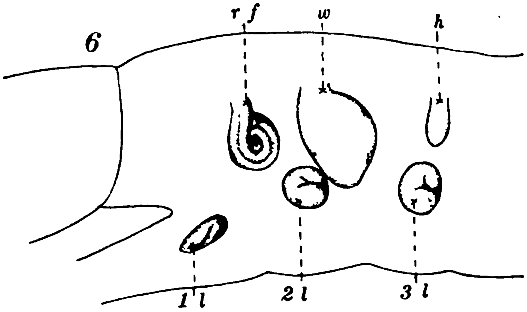

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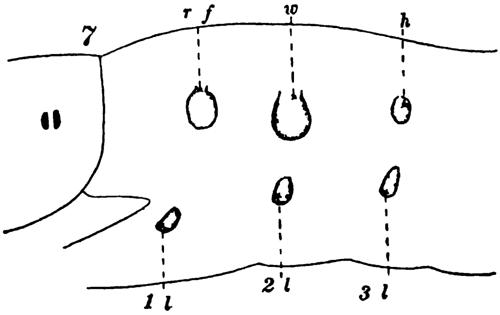

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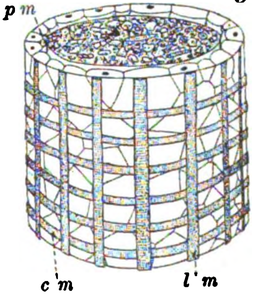

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